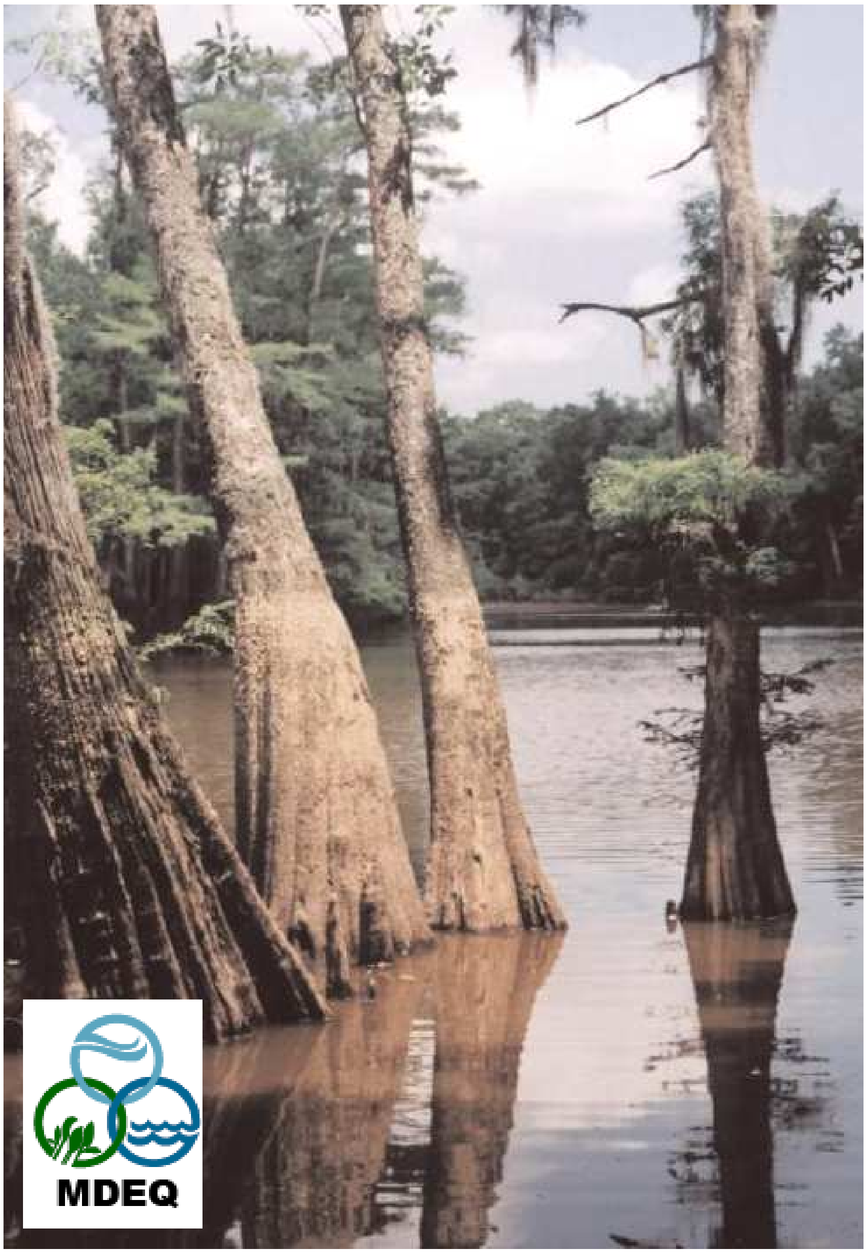
# Revised Draft Nutrient Thresholds to Protect Aquatic Life Uses in Mississippi Non-Tidal Streams and Rivers




Pascagoula River        Photo credit: Kirk Loy

**June 8, 2011**

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**Prepared for:**

**Mississippi Department of Environmental Quality**
**Office of Pollution Control**
**2380 Highway 80 West**
**Jackson, Mississippi 39204**

**Prepared by:**

**Tetra Tech, Inc.**
**Center for Ecological Sciences**
**400 Red Brook Blvd., Suite 200**
**Owings Mills, Maryland 21117-5159**

**2010/2011 Document Revision: Contractual Agreement Number MDEQ-06-ID-01TTbetween Mississippi Department of Environmental Quality (MDEQ) and Tetra Tech, Inc.; MDEQ Work Order Nos. 10-01TT-22 and 11-01TT-28, Tetra Tech Project Nos. 25989 and 27662**

**June 8, 2011**

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# Acknowledgements

This revision of the stream nutrient threshold report was completed for the state of Mississippi Department of Environmental Quality (MDEQ) under Contractual Agreement Number MDEQ-06-ID-01TT between Mississippi Department of Environmental Quality (MDEQ) and Tetra Tech, Inc. (MDEQ Work Order Nos. 10-01TT-22 and 11-01TT-28, Tetra Tech Project Nos. 25989 and 27662). The primary authors are Lei Zheng and Michael J. Paul of Tetra Tech, Inc., Center for Ecological Sciences in Owings Mills, MD. Catherine Carter provided editorial assistance. Review and feedback on the original report (MDEQ 2009) was provided by Kim Caviness, Natalie Segrest, and Valerie Alley. In addition, the MDEQ Nutrient Technical Advisory Group provided feedback that served as the basis for the reanalysis in this report. All field work was performed by MDEQ staff.

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# Executive Summary

In response to the threat posed by nutrients, EPA requested that states develop criteria to protect designated uses from impairment due to excessive nutrients. The State of Mississippi implemented this project to support development of nutrient criteria for non-tidal wadeable and non-wadeable streams within the State. EPA recommended three methods to establish nutrient criteria (USEPA 2000): a frequency distribution reference-based approach, a stressor-response approach, and literature-derived values. In the original report (MDEQ 2009), we used a weight of evidence approach, combining these three methods to derive nutrient thresholds from which final recommended criteria may ultimately be selected. The original report was reviewed by the Mississippi Department of Environmental Quality (MDEQ) Nutrient Criteria Technical Advisory Group (TAG). They provided feedback and suggested analyses. In addition, MDEQ had collected additional data since the original report was written. This report incorporated the new data, conducted the original analyses again, and incorporated new analyses. The intent is not to supplant the original report, but to supplement that report with this new one. Readers are encouraged, therefore, to consider both reports as complementary and relevant.

As with the original report, we collected and compiled data for streams in Mississippi available from seven different sources incorporating updated MDEQ monitoring data. These datasets included nutrients and other related water quality parameters, as well as biological assemblage information, i.e., algal, benthic macroinvertebrate, and fish biomass and composition. Appropriate QA/QC was further performed to assess the quality of the data and condense the data into three separate datasets for wadeable streams [the Mississippi Benthic Index of Stream Quality (M-BISQ) project dataset, Mississippi Department of Environmental Quality (MDEQ) WADES database dataset, and a combined M-BISQ and WADES dataset. Due to a limited number of algal data, macroinvertebrate data for M-BISQ development was used as the primary biological response data for stressor response analyses. Other datasets were used to derive benchmarks using frequency distribution reference approaches.

As in the original report, we classified streams in the State based on bioregional classification to reduce variability. Preliminary analysis indicated that bioregional classification provided better resolution than level III ecoregions. Also, bioregional classification provided more reference sites for ecoregion 75 and thus strengthened threshold development for this region. The most important advantage was that biological thresholds (i.e., M-BISQ scores) have been determined for evaluating biological condition in these bioregions, so stressor values could be linked to direct measures of designated uses in each bioregion.

Three reference condition groups were defined following Stoddard et al. (2006). The minimally disturbed condition (MDC), representing the estimated condition in the absence of human disturbance, was estimated using regression equations of nutrient concentrations against human land uses across watersheds and extrapolating to zero human land use conditions (Dodds and Oaks 2004). The least disturbed condition (LDC) represents a baseline that should protect assigned designated uses and is characterized by least disturbed watersheds in a region. For this revised thresholds document, LDC was defined using the same reference site selection criteria used to develop the M-BISQ, which was based on regional land use, stream physical habitat, and chemical characteristics, except we excluded nutrient variables to avoid circularity. Because information about the LDC was not available for a dataset, we used a second LDC method: the 25th percentile of a distribution of samples from the entire population of waterbodies within a given physical classification, which served as a surrogate for the 75th percentile of a sample distribution from LDC sites (USEPA 2000a). The third reference condition set, best attainable condition (BAC), is defined as that population of sites known to be exceeding acceptable thresholds for biological condition based on MBISQ macroinvertebrate index scores and was defined using the biological thresholds defined by M-BISQ scores for each bioregion (lower quartile of reference site M-BISQ07 scores).

Nutrient benchmarks derived from different reference approaches varied across different bioregions. Generally speaking, nutrient thresholds of MDC derived from land use extrapolation were lower than those derived from LDC and BAC conditions. Although reference distribution based approaches typically employ the LDC, it was restricted by the availability of sites in some regions. Benchmarks of BAC were similar to that of LDC in most regions, and in some cases were higher than LDC.

Stressor-response relationships are a critical part of threshold development as they can provide direct linkages between nutrients and use measures. Algal biomass (Chl *a* in water column) in streams did not respond to elevated nutrient concentrations. However, macroinvertebrate metrics did. Nutrients affect macroinvertebrates indirectly through a number of pathways including oxygen declines due to excess algal growth and heterotrophic respiration from enrichment and excess and nuisance algal growth from enrichment reducing habitat and food quality. However, other stressors also affect macroinvertebrates, some of which can co-occur with nutrients, such as suspended sediment, especially when using field derived data. It is important, therefore, to provide as much support as possible of a presumptive causal linkage between nutrients and invertebrates. One approach for investigating the independent effect of nutrients in amidst covarying stressors is with propensity score analysis (USEPA 2010). This approach evaluates nutrient invertebrate responses after mitigating the effects of covariates by creating bins with similar covariate distributions. Propensity score analysis indicated effects of covariates weakened relationships between nutrients and response variables, but these effects were still evident and significant after adjusting for their effects. We conclude that stressor-response relationships consistent with a presumed effect of nutrients on invertebrates exist and can be used as the basis for deriving thresholds. Having supported this causal linkage, we then conducted stressor-response analyses.

USEPA recently revised guidance on methods for evaluating stressor-response relationships for deriving nutrient criteria (USEPA 2011). We revised our analytical approach in light of the new guidance. Having already undertaken classification, data were explored, significant correlations were identified between macroinvertebrate metrics and nutrient parameters, and the nature of the relationships (linear or non-linear) were explored and identified. We then applied interpolation for linear relationships and change-point analysis using nonparametric deviance reduction and split-point regression for non-linear relationships to identify thresholds (Qian et al. 2003). We applied these to raw data as well as probabilities of exceeding biological thresholds estimated with logistic regression. For all analyses we calculated mean thresholds as well as confidence intervals using resampling techniques. For this revised report, we had sufficient sample size to evaluate the western region as both a northern (ecogroup 1) and southern (ecogroup 5) subregion. Values for TN and TP based on this revised stressor-response analysis were comparable to values generated for the original stream report.

Literature derived nutrient thresholds were not updated from the original report and recommended endpoints from that analysis have not changed.

The different approaches resulted in similar but distinct nutrient thresholds across the various classes of Mississippi wadeable streams. The tables below indicate recommended thresholds for TN and TP, respectively, as well as ranges (in parentheses) based on uncertainty analyses conducted for the various analyses. The range provided for literature values represents the range in reported values from those studies, which include actual or proposed state regulatory thresholds in the southeast (Tennessee, Alabama, Florida, Kentucky, and a USGS study) and from other regions around the US.

<table>
<tr><th rowspan="3"></th><th colspan="6">TN (mg/L)</th></tr>
<tr><th rowspan="2">Modeled Reference (Land Use)</th><th colspan="2">Distribution Based</th><th rowspan="2">Stressor-Response</th><th rowspan="2">Regional State Values</th><th rowspan="2">Other Regions</th></tr>
<tr><th>Least Disturbed</th><th>Biologically Attaining</th></tr>
<tr><td>East</td><td>0.360<br>(0.240-0.540)</td><td>0.640<br>(0.600-0.700)</td><td>0.740<br>(0.680 – 0.800)</td><td>0.850<br>(0.650-0.960)</td><td rowspan="6">0.510 – 1.87</td><td rowspan="6">0.180 – 0.197</td></tr>
<tr><td>South Bluff</td><td>0.380<br>(0.170-0.450)</td><td>0.460<br>(0.380-0.720)</td><td>0.610<br>(0.450-0.760)</td><td>N/A</td></tr>
<tr><td>West</td><td>0.240<br>(0.160-0.370)</td><td>0.780<br>(0.780-0.930)</td><td>0.940<br>(0.940-1.110)</td><td></td></tr>
<tr><td>West: North (ecogroup 1)</td><td colspan="3" rowspan="2"></td><td>1.23<br>(0.640-2.370)</td></tr>
<tr><td>West: South (ecogroup 5)</td><td>0.470<br>(0.240-0.900)</td></tr>
<tr><td>Southeast</td><td>0.380<br>(0.260-0.560)</td><td>0.580<br>(0.540-0.710)</td><td>0.680<br>(0.670-0.810)</td><td>0.310<br>(0.140-0.730)</td></tr>
</table>

<table>
<tr><th rowspan="3"></th><th colspan="6">TP (mg/L)</th></tr>
<tr><th rowspan="2">Modeled Reference (Land Use)</th><th colspan="2">Distribution Based</th><th rowspan="2">Stressor-Response</th><th rowspan="2">Regional State Values</th><th rowspan="2">Other Regions</th></tr>
<tr><th>Least Disturbed</th><th>Biologically Attaining</th></tr>
<tr><td>East</td><td>0.020<br>(0.010-0.040)</td><td>0.050<br>(0.040-0.050)</td><td>0.050<br>(0.040 – 0.060)</td><td>0.060<br>(0.050-0.070)</td><td rowspan="6">0.020 – 0.500</td><td rowspan="6">0.020-0.200</td></tr>
<tr><td>South Bluff</td><td>0.070<br>(0.050-0.100)</td><td>0.130<br>(0.080-0.160)</td><td>0.110<br>(0.080-0.160)</td><td>N/A</td></tr>
<tr><td>West</td><td>0.030<br>(0.020-0.050)</td><td>0.110<br>(0.090-0.140)</td><td>0.100<br>(0.080-0.120)</td><td></td></tr>
<tr><td>West: North</td><td colspan="3" rowspan="2"></td><td>0.120<br>(0.050-0.300)</td></tr>
<tr><td>West: South</td><td>0.040<br>(0.020-0.090)</td></tr>
<tr><td>Southeast</td><td>0.010<br>(0.010-0.020)</td><td>0.030<br>(0.020-0.040)</td><td>0.050<br>(0.040-0.050)</td><td>0.040<br>(0.020-0.060)</td></tr>
</table>

# 1 Introduction

Nutrients are a natural component of healthy ecosystems. In natural concentrations, essential nutrients help maintain the structure and function of ecosystems. However, in excessive quantities, nutrients can destabilize natural ecosystems leading to a variety of problems including nuisance plant growth, hypoxia and anoxia, species loss, and risks to human health.

Nutrients affect aquatic systems in diverse ways. The direct effects are on the primary producers, namely, algal and macrophyte production and species composition. The effects on most non-primary producer aquatic life are indirect (Figure 1.1).

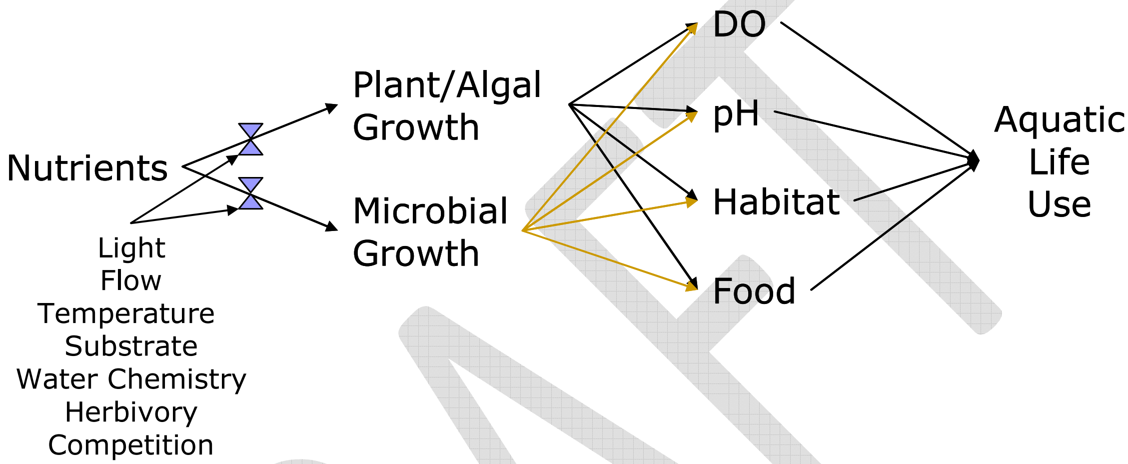


**Figure 1.1 Simplified diagram illustrating the causal pathway between nutrients and aquatic life use impacts. Nutrients enrich both plant/algal as well as microbial assemblages, which lead to changes in the physical/chemical habitat and food quality of streams. These effects directly impact insect and fish assemblages. The effects of nutrients are influenced by a number of other factors as well, such as light, flow, and temperature.**

Nutrients increase the growth of primary producers and decomposers which lead to changes in the physical and chemical stream environment (e.g., reduced oxygen, loss of reproductive habitat, alteration of the food base for aquatic animals, etc.). It is these effects which result in changes to the biological stream community (e.g., loss of disturbance sensitive taxa), and ultimately impair the use of a stream for aquatic life.

In response to the threat by nutrients, EPA has requested that states develop nutrient criteria to protect designated uses from impairment due to excessive nutrients. Nutrient criteria are developed to protect designated uses and, as such, the applicable designated uses are integral to guiding the appropriate criteria. Nutrients principally threaten aquatic life, recreational, and drinking water uses. Aquatic life uses are threatened when nutrients actually impair plant communities and result in the proliferation of nuisance or invasive taxa or cause excessive growth of algae, which alters the habitat (physical habitat, dissolved oxygen, etc.) for other aquatic life. Recreational uses are threatened when nutrients cause growth of plant taxa that interfere with fishing, swimming, or other recreational uses of streams and rivers. Lastly, drinking water uses are impaired when nutrients cause the proliferation of taxa that generate taste and odor problems in drinking water, produce toxic compounds, or, potentially, overwhelm filtration systems.

EPA has developed recommended regional nutrient criteria, but encouraged states to pursue their own nutrient criteria development programs. The state of Mississippi has committed to the development of scientifically defensible nutrient criteria to protect designated uses in its waterbodies. As such, MDEQ developed nutrient thresholds for streams as part of an earlier effort (MDEQ 2009). In response to additional data collection and feedback from the MDEQ nutrient Technical Advisory Group (TAG), additional data incorporation and analyses were recommended. This report summarizes those additional efforts. Some text was carried forward from the original report to provide appropriate context, but readers are encouraged to consult the original report for a complete understanding of the data and analyses conducted.

# 2 Current status of nutrient criteria for streams and rivers in Mississippi

The U.S. Environmental Protection Agency (EPA), in its recommendations for nutrient criteria development, specified that "ecoregional nutrient criteria will be developed to account for the natural variation existing within various parts of the country" (USEPA, 2000). They go on to explain the importance of ecoregions:

*"Ecoregions serve as a framework for evaluating and managing natural resources. The ecoregional classification system developed by Omernik (1987) is based on multiple geographic characteristics (e.g., soils, climate, vegetation, geology, land use) that are believed to cause or reflect the differences in the mosaic of ecosystems."*

Ecoregions denote areas of general similarity in ecosystems and in the type, quality, and quantity of environmental resources. They are designed to serve as a spatial framework for the research, assessment, management, and monitoring of ecosystems and ecosystem components. There are 4 level III ecoregions [Southeastern Plains (65), Mississippi Alluvial Plain (73), Mississippi Valley Loess Plains (74), and Southern Coastal Plain (75)] and 21 level IV ecoregions in Mississippi (Figure 2.1), and most continue into ecologically similar parts of adjacent states. The ecological and biological diversity within Mississippi is vast. The state contains barrier islands and coastal lowlands, large river floodplain forests, rolling and hilly coastal plains with evergreen and deciduous forests, and a variety of aquatic habitats (ftp://ftp.epa.gov/wed/ecoregions/ms/ms_eco.html). Since the Mississippi Alluvial Plain (73), or Delta, has special geographic and land use patterns, nutrient criteria development at this stage does not include this particular ecoregion.

MDEQ conducted statewide biological monitoring using benthic macroinvertebrates as an indicator of biological integrity for wadeable streams (MDEQ, 2003a). The primary intent of this effort was the development of a credible and scientifically-defensible biological assessment tool to be used in the assessment of Mississippi's wadeable streams and rivers, the Mississippi Benthic Index of Stream Quality (M-BISQ). This index was then used in the biological assessment of the State's wadeable streams and rivers.

Recently, MDEQ conducted a new analysis to recalibrate the M-BISQ (MDEQ 2007a). In this round of analysis, the State was divided into 4 different bioregions according to macroinvertebrate assemblages (Figure 2.1). These bioregions encompass 7 different ecogroups with different environmental characteristics. At the same time, biological indicators were also developed for non-wadeable streams (MDEQ 2007b). These efforts will be useful for linking nutrient impairment to biological criteria. To protect biological integrity within each bioregion, a corresponding nutrient criterion would be established.

Currently, the state of Mississippi has no numeric criteria for total nitrogen and phosphorus. Two nutrient compounds are regulated by the State Water Quality Standards (WQS): ammonia and nitrate (MDEQ 2003b). Ammonia can be potentially toxic to aquatic life under different pH and temperature levels and Mississippi uses the USEPA recommended ammonia criteria to protect aquatic life. Nitrate concentration above 10 mg/L is associated with increased risk of sickness (e.g., methemoglobinemia) in human infants. As a result, the human health criterion for nitrate is

10 mg/L for public water supply. In addition to these nutrient compounds, Mississippi's WQS also contain turbidity and dissolved oxygen criteria for all waterbodies. Furthermore, MDEQ (2004) has developed a nutrient criteria development plan for waters within the State and has completed draft lake nutrient thresholds analysis (MDEQ 2007e).

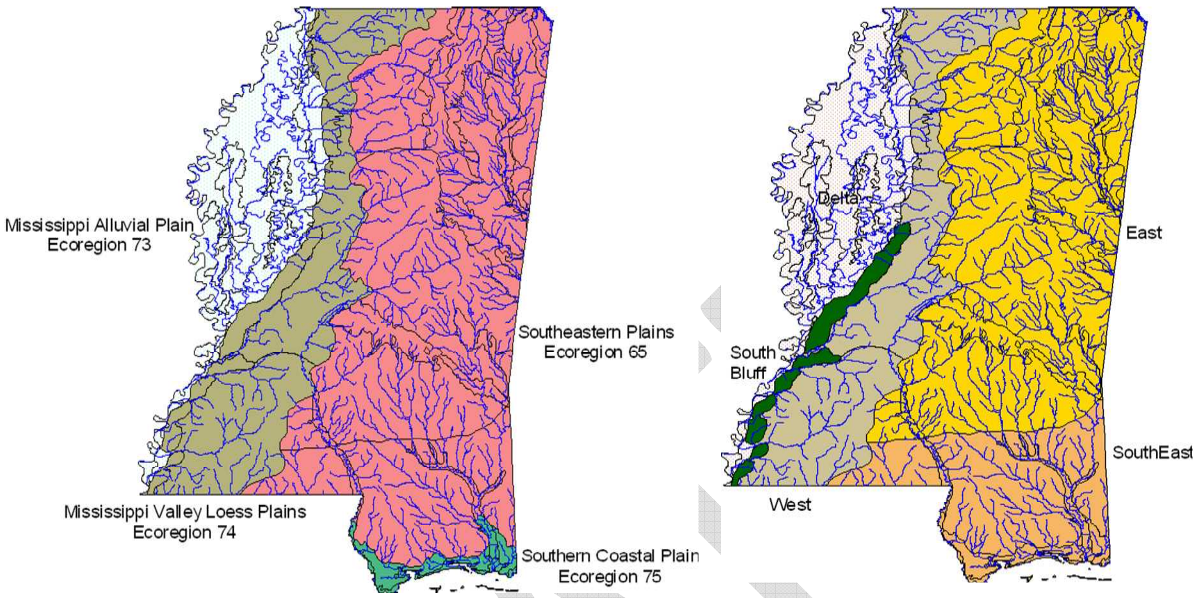


**Figure 2.1 Major ecoregions and bioregion within the State of Mississippi.**

In addition to existing state water quality criteria, the USEPA has also recommended regional nutrient criteria for ecoregions in Mississippi. USEPA aggregated level III ecoregions into relatively homogeneous nutrient ecoregions according to background nutrient concentrations. The three level III ecoregions in Mississippi (excluding ecoregion 73) were aggregated into one single nutrient ecoregion: Region IX (Southeastern Temperate Forested Plains and Hills). The recommended nutrient criteria for streams in this region are: TP 37 µg/L and TN 0.69 mg/L (USEPA 2000b).

Nutrient TMDLs have also been developed for individual basins (e.g., Pascagoula watershed). A preliminary study to develop TP targets for the Pascagoula basin in Mississippi has been conducted and proposed (RMA 2005). Although this study primarily focused on the Pascagoula basin and utilized only 2001 M-BISQ data, it examined the statewide nutrient classification strategies and developed a TP target using a reference-based approach. A TP concentration of 0.07 – 0.11 mg/l was proposed as the preliminary target.

Finally, in order to strengthen the scientific defensibility and protectiveness of their nutrient criteria, MDEQ has gathered a panel of the recognized state water quality experts into the Nutrient Criteria Technical Advisory Group (TAG) that is providing scientific oversight and recommendations to MDEQ in support of nutrient criteria development. This document is in response to TAG review and feedback on the original draft threshold document for streams and the group is similarly engaged in providing technical review for all other waterbody types (MDEQ 2007e).

# 3 Data preparation and analytic approaches

## 3.1 Sampling locations and land use analysis

Over 850 unique river and stream sampling stations across the entire state of Mississippi were sampled for physical habitat, chemical parameters, and benthic macroinvertebrate composition (Figure 4.1) from 2001 to 2009. These stations are distributed across four different ecoregions and six biological regions. The original report only considered data through 2005.

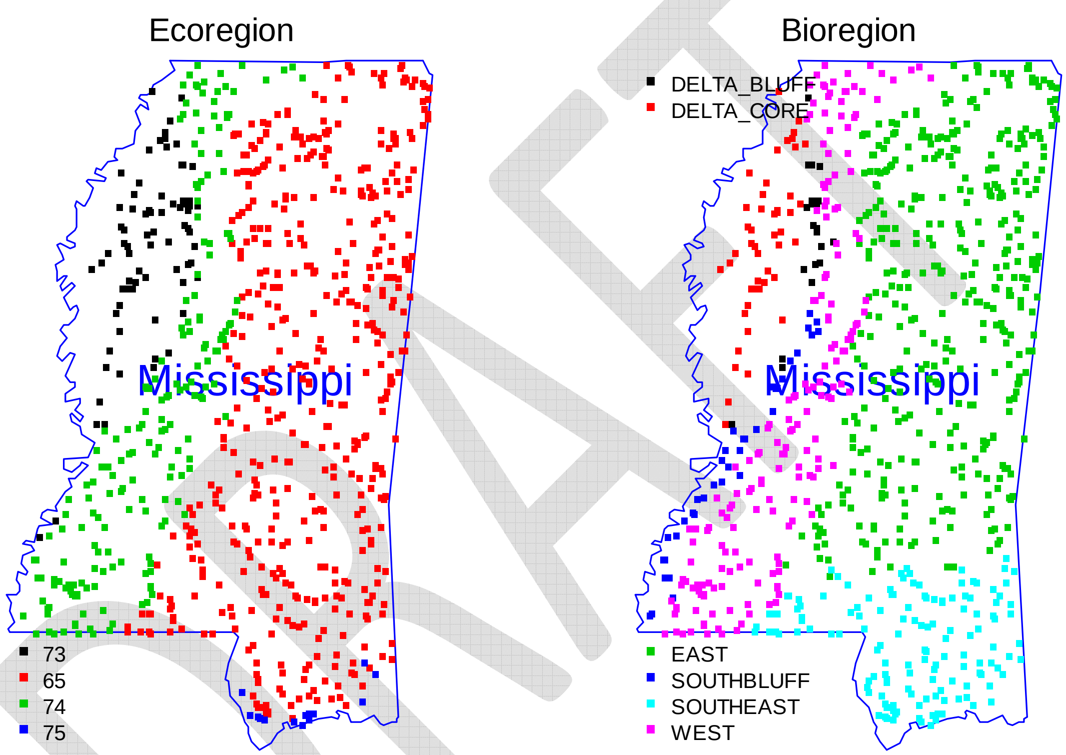


**Figure 3.1 Sampling locations in each ecoregion and bioregion within the State of Missisippi**

According to EPA level 3 ecoregion classification, the four ecoregions within the State have different geological and land uses characteristics, as well as climate conditions (Omernik et al. 2008).

*65. SOUTHEASTERN PLAINS*
*These irregular plains have a mosaic of cropland, pasture, woodland, and forest. Natural vegetation was predominantly longleaf pine, with smaller areas of oak-hickory-pine and Southern mixed forest. The Cretaceous or Tertiary-age sands, silts, and clays of the region contrast geologically with the older metamorphic and igneous rocks of the Piedmont (45), and with the Paleozoic limestone, chert, and shale found in the Interior Plateau (71). Elevations and*

*relief are greater than in the Southern Coastal Plain (75), but generally less than in much of the Piedmont. Streams in this area are relatively low-gradient and sandy-bottomed.*

*73. MISSISSIPPI ALLUVIAL PLAIN*
*This riverine ecoregion extends from southern Illinois, at the confluence of the Ohio River with the Mississippi River, south to the Gulf of Mexico. It is mostly a broad, flat alluvial plain with river terraces, swales, and levees providing the main elements of relief. Soils are typically finer-textured and more poorly drained than the upland soils of adjacent Ecoregions 35 and 74, although there are some areas of coarser, better-drained soils. Winters are mild and summers are hot, with temperatures and precipitation increasing from north to south. Bottomland deciduous forest vegetation covered the region before much of it was cleared for cultivation. Presently, most of the northern and central parts of the region are in cropland and receive heavy treatments of insecticides and herbicides. Soybeans, cotton, and rice are the major crops.*

*74. MISSISSIPPI VALLEY LOESS PLAINS*
*This ecoregion stretches from near the Ohio River in western Kentucky to Louisiana. It consists primarily of irregular plains, some gently rolling hills, and near the Mississippi River, bluffs. Thick loess is one of the distinguishing characteristics. The bluff hills in the western portion contain soils that are deep, steep, silty, and erosive. Flatter topography is found to the east, and streams tend to have less gradient and more silty substrates than in the Southeastern Plains ecoregion (65). To the east, upland forests dominated by oak, hickory, and both loblolly and shortleaf pine, and to the west on bluffs some mixed and southern mesophytic forests, were the dominant natural vegetation. Agriculture is now the typical land cover in the Kentucky and Tennessee portion of the region, while in Mississippi there is a mosaic of forest and cropland.*

*75. SOUTHERN COASTAL PLAIN*
*The Southern Coastal Plain consists of mostly flat plains, but it is a heterogeneous region containing barrier islands, coastal lagoons, marshes, and swampy lowlands along the Gulf and Atlantic coasts. In Florida, an area of discontinuous highlands contains numerous lakes. This ecoregion is lower in elevation with less relief and wetter soils than the Southeastern Plains (65). It is warmer, more heterogeneous, and has a longer growing season and coarser textured soils than the Middle Atlantic Coastal Plain (63). Once covered by a variety of forest communities that included trees of longleaf pine, slash pine, pond pine, beech, sweetgum, southern magnolia, white oak, and laurel oak, land cover in the region is now mostly slash and loblolly pine with oak-gum-cypress forest in some low lying areas, citrus groves in Florida, pasture for beef cattle, and urban.*

In order to evaluate the impact of human land cover on the nutrient concentrations in streams, land cover was calculated using a GIS analysis conducted by MDEQ. Land uses were discerned in seven categories using National Land Cover Database (NLCD) 2001 data as the source. The categories included water, forest, wetland, pasture/grass, cropland, scrub/barren, and urban. Water, forest, and wetland areas were considered natural land uses and the rest were considered human land use.

Land cover in areas upstream of the sampling location were delineated in four spatial arrangements (Figure 3.2): land cover in the whole catchment above the sampling location, land cover in the 100m stream buffer zone along all streams in the whole catchment above the sampling location, land cover in the 100m stream buffer zone around all streams within 1 km above the sampling location only, and land cover in the 50m stream buffer zone around all streams within1 km above the sampling location only. Delineation was automated and corrected as needed so that sampling location coordinates always fell in the appropriate stream channel. USGS 12-digit subwatersheds were the starting point for upstream delineations; by cutting the subwatershed containing the sampling point along ridges determined by MDEQ 10 meter digital elevation models (DEMs), the near site drainage was delineated. All subwatersheds upstream from this were then selected and merged to delineate entire catchments.

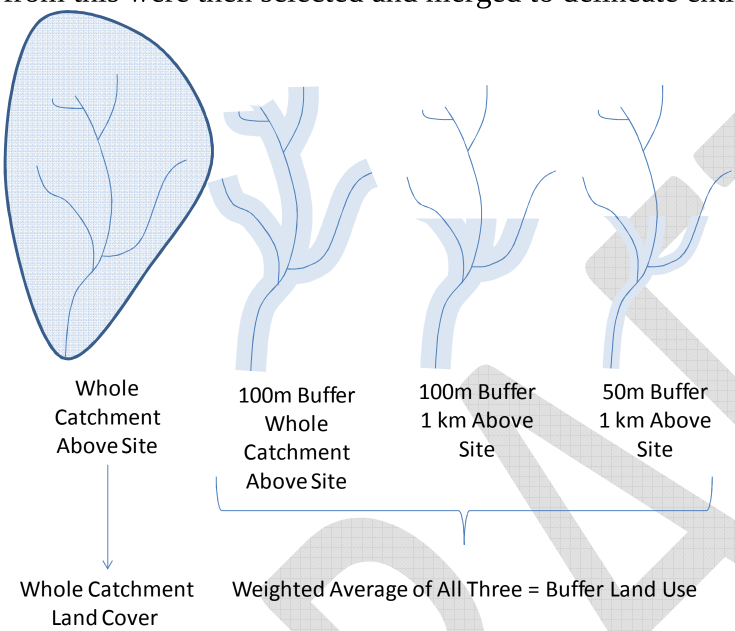


**Figure 3.2 Schematic of land cover characterization approaches.**

To simplify the analysis, land cover characteristics upstream of each sampling location were summarized in two ways: for the whole catchment estimate as a straight percentage of land cover and for the buffer land cover as a weighted average of percentages across the three buffer zones. The weighting was accomplished by averaging the land use percentages over the three buffer zones. The closest buffer zone (less than 1km from the site and less than 50 meters from the stream) is part of all three buffer zones and is thus included three times in a simple average, giving it more weight than the buffer zones of intermediate (within 100m of a stream also within 1km of point outside of 100m buffer) or longest extent (within 100m of a stream throughout the whole upstream catchment). Likewise, the intermediate buffer zone is part of the larger buffer zone and is therefore double-counted when included in the average. Land uses in the 100 m buffer more than 1 km upstream were only included with the largest buffer zone and therefore carry less weight in the average.

## 3.2 Physical and chemical variables

Data from a total of 705 discrete river and stream sampling stations across the State of Mississippi (excluding 79 stations in the Mississippi Alluvial Plain or MS Delta) were used. These stations represented a range of geographic location, non-point source pollution from urban, agricultural, and silviculture lands as well as point source pollution from NPDES permitted facilities, and biological condition.  Data included:

- Qualitative (visual observations) habitat assessment scores.  Data were collected in winter from 2001 to 2009 according to MDEQ Standard Operating Procedures (2007c) and are described in detail in the Quality Assurance Project Plan (QAPP) for 303(d) List

Assessment and Calibration of the Index of Biological Integrity for Wadeable Streams in Mississippi (MDEQ, 2001). Ten habitat parameters describing instream habitat, bank, and riparian conditions were visually assessed and rated on a scale from 0 to 20 with 0 being the poorest habitat and 20 being optimal. Habitat assessments were performed on the same 100-meter reach from which macroinvertebrate samples were collected. Duplicate and repeat habitat assessments were performed at 70 randomly chosen sites. Individual habitat parameters were also summed into three subcategories describing stream characteristics including in-stream, morphological, and riparian habitat conditions. Sediment particle size was measured using a modified 100-particle Wolman pebble count (MDEQ, 2001). Resulting data are presented as the percent of silt/clay, sand, gravel, cobble, boulder, and/or hardpan to total particle size.

- Physicochemical measurements. Field physicochemical data (dissolved oxygen, pH, temperature, specific conductance, TDS, and turbidity) were collected using a multi-probe and turbidimeter. Water chemistry grab samples were collected at the same time macroinvertebrate samples were collected. These data were collected in the winter from 2001-2009 (one sampling event taken at the time of biological sample collection) and the spring and fall of 2004 (spring season = mid-March through April, fall season = mid-August through September). The 2004 data collection strategy included two sampling events in the spring season and two sampling events in the fall season. The most recent chemistry sample, which has a correspondent macroinvertebrate sample from each station, was selected to represent the most contemporaneous environmental condition. Sampling times varied from 7 am through 6 pm. Nutrient parameter concentrations included ammonia, total kjeldahl nitrogen (TKN), nitrate + nitrite, total nitrogen (TN = TKN + nitrate/nitrite), orthophosphate, and total phosphorus (TP). Data were collected according to MDEQ Standard Operating Procedures and are described in detail in the Quality Assurance Project Plan (QAPP) for 303(d) List Assessment and Calibration of the Index of Biological Integrity for Wadeable Streams in Mississippi (MDEQ, 2001). Procedures used to conduct laboratory analysis of water samples for various physical and chemical water quality parameters were also performed as noted in the QAPP according to MDEQ Analytical Chemistry Lab Methods (MDEQ, 2001). Various physical and chemical parameters were measured using EPA-approved methods.

We applied the following rules to control the quality of our datasets:

1. Method detection limits (MDLs) were frequently reported and samples flagged if they were below the MDL in the original datasets. Values below MDLs were analyzed as half of the MDL, which is one of the most common practices for similar statistical analysis;
2. The primary variables considered for nutrient threshold development were water column concentrations of TN, TP, water column and benthic algal biomass as chl *a*, and turbidity. Due to the lack of benthic algal biomass measurements, only TN, TP and turbidity were gathered during data collection/database building. TN and TP are the primary causal variables most closely related to response variables in streams. Nitrate plus nitrite and orthophosphate were also considered in our analysis. However, due to a lack of strong correlation between these two variables and biological responses, they

were not considered for threshold development. TN and TP were log-transformed in most circumstance in order to obtain normally distributed data. Algal biomass, as represented by chlorophyll *a* and turbidity, was also log-transformed. Although turbidity is not commonly used as an index of eutrophication in streams, it nonetheless should increase in streams with increasing algal biomass due to nutrient enrichment; it was also log-transformed.

## 3.3 Benthic macroinvertebrate assemblages

Macroinvertebrate data were collected in the winter of 2001-2009 and samples were processed according to MDEQ Standard Operating Procedures and are described in detail in the Quality Assurance Project Plan (QAPP) for 303(d) List Assessment and Calibration of the Index of Biological Integrity for Wadeable Streams in Mississippi (MDEQ, 2001). Benthic macroinvertebrate specimens were identified, tallied, and recorded. The benthic taxonomic information was recorded and stored in a database (M-BISQ database), along with associated habitat, chemistry, and land use data for over 700 sites.

Indices employing macroinvertebrates are reliable and frequently used for water quality assessment (Barbour et al. 1999, USEPA 2000a, Davies and Jackson 2006). Benthic macroinvertebrate community metrics, including the overall M-BISQ score, were calculated in the MDEQ EDAS database (MDEQ 2007c). Over 60 different biological metrics describing various characteristics of the macroinvertebrate assemblage were derived from the taxonomic data. A suite of regionally specific metrics were used to calculate an overall M-BISQ score according to methods outlined in the M-BISQ QAPP (MDEQ, 2001). Tetra Tech, Inc recently updated and recalculated the M_BISQ for the state of Mississippi (MDEQ 2007a).

Individual macroinvertebrate taxa respond to enrichment, and some are particularly sensitive. Individual metrics, such as EPT taxa, were used as response variables. The richness metrics were log-transformed if necessary and percent metrics were arcsine square-root transformed. M-BISQ scores were standardized values and were not transformed in most circumstances.

## 3.4 Analytical approaches

Traditionally, water quality criteria to protect aquatic life use were developed using toxicological approaches. Such approaches have been applied for a range of pollutants to develop water quality criteria. However, as explained above, nutrient enrichment does not have a direct toxicological effect. Nutrients, however, directly alter the diversity and composition of algal and plant aquatic life, and stimulate primary production and heterotrophic respiration. For insects, fish, and other aquatic life, the mode of action of nutrients is indirect and through a causal pathway that involves alteration of their physical, chemical, and biological environment as a result of the effects on primary producers and heterotrophs described above (Figure 1.1). As a result, traditional toxicological approaches are not appropriate.

EPA has recommended three methods to establish nutrient criteria (USEPA 2000): a reference-based approach, a stressor-response approach, and literature-derived values. The reference-based approach is represented by two basic methods that attempt to estimate that condition occurring in relatively unimpacted or historic reference sites, the assumption being that such conditions are implicitly supportive of designated uses. As such, nutrient concentrations associated with those conditions ought to be protective. The first reference method derives criteria from the distribution of ambient conditions in a population of reference sites. This first method has been commonly used to develop biocriteria and nutrient criteria, including EPA recommended regional nutrient criteria (Dodds et al. 1998, USEPA 2000b, Seip et al. 2000, Dodds and Welch 2000, Rohm et al. 2002, Ice and Binkley 2003). A second reference method is to estimate reference conditions by empirical modeling either through land cover – nutrient concentration models solved for the condition of zero percent human land cover (Dodds and Oaks 2004), or building reference condition regression models based on multiple natural predictors (Smith et al. 2003, Sheeder and Evans 2004). Either reference approach method requires appropriate classification in order to establish suitable criteria for different waterbodies (Detenbeck et al. 2004, Snelder et al. 2004, Wickham et al. 2005).

The use of stressor-response relationships is a second method for deriving nutrient criteria (USEPA 2000a, 2010). It has been argued that reference approaches using a percentile of ambient nutrient concentrations within a waterbody class alone to establish criteria can lack a direct linkage to designated use protection (Dodds and Welch 2000, McMahon et al. 2003). Aquatic life uses are one of the uses most commonly targeted for protection by nutrient criteria, and stressor-response approaches derive criteria based on relationships between aquatic life measures and nutrient concentrations. Biological assessment has been shown to be an efficient way to protect aquatic life uses (Barbour et al. 1996, 1999, 2000, King and Richardson 2003), and the indicators that are developed provide a direct measure of aquatic life use condition. As a result, correlation or regression analyses that directly relate eutrophication stressors to biological indicators or other valued aquatic life use attributes provide strong justification for ecologically meaningful criteria. Establishment of nutrient criteria using stressor-response approaches have relied on algal biomass and algal community indicators, among others (Welch 1988, Stevenson 1997, Biggs et al. 2000, Havens 2003). In addition, experimental approaches have been used to establish or verify the cause and effect relations between algal assemblages and potential nutrient endpoints (Havens and Aumen 2000). There is no reason that other response measures related to other uses could not also be used in such an analytical framework. For instance, indicators derived from recreational user perception surveys can also be related to nutrient concentrations and stressor response analysis used to develop criteria that protect recreational use.

The third approach is based on deriving criteria from existing literature for the same or similar regions. This approach recognizes that the limnology with regards to nutrient impacts of many systems has been well investigated in the research literature and that this literature provides another important source of guidance in developing protective nutrient criteria. This approach also includes the use of mechanistic models to develop nutrient criteria. In many regions, nutrient data are limited or not available, and interactions among multiple factors are difficult to incorporate into statistical models. In this case, mechanistic modeling approaches can be applied to establish water quality criteria for many streams and lakes (Somlyody 1997, 1998, Dodds et al. 2002, Reckhow et al. 2005). The modeling approach has been principally used for site specific criteria, since site specific predictors are generally used.

We used a weight of evidence approach that incorporates all three approaches to develop recommended nutrient thresholds. The weight of evidence approach is actually the recommended strategy for states to develop scientifically defensible criteria (USEPA 2000). The endpoints derived from each method are weighed for the strength of each analysis, based on data quality and relevance, using professional judgment. A final recommended threshold is selected that balances these weights with the provision that the threshold is assured to protect the use, and the process is documented so as to be transparent.

# 4 Classification

Classification was discussed in detail in Appendix C of the original report (MDEQ 2009) and, for brevity, is not repeated here.

# 5 Reference based approaches to develop nutrient thresholds

The “reference site approach” (Hughes 1995, Bailey et al. 2004) was originally developed to quantify the biological condition at a set of sites that are either minimally or least disturbed by human activity. This approach is the most common approach for estimating the various reference states and is a scientifically sound method for setting expectations, provided that the form of reference condition that the reference sites represent is clearly defined. These reference states fall within the biological condition gradient as described by Davies and Jackson (2006).

Stoddard et al. (2006) described various reference condition definitions and called for consistency in use of the term “reference condition”. In this paper, they defined reference condition of biological integrity (RC-BI) as the natural biological condition that is ideal but may never be attainable. Least disturbed condition (LDC) is a preferred term for a set of criteria that, in total, describe the characteristics of sites in a region that are the least exposed to stressors; this is also the most widely applied approach. We introduce an additional reference population, the biologically healthy condition (BHC) as those sites currently exceeding MBISQ index thresholds for acceptable biological condition.

In this study, we used three reference approaches: 1) Extrapolating reference conditions from empirical models of land cover against nutrients to estimate natural conditions; 2) LDC reference sites defined by human land uses and disturbance, and 3) BHC reference sites defined by M-BISQ thresholds for each bioregion. Again, we introduced the BHC concept and defined it as all sites that currently exceed MBISQ index thresholds for acceptable biological condition.

## 5.1 Thresholds from estimating natural background concentrations

Dodds and Oakes (2004) proposed a regression approach to estimate reference conditions by extrapolating nutrient concentrations to those existing under no human land cover disturbance. MDEQ (2009) also adopted the same approach for extrapolating nutrient concentrations in streams under natural conditions for different ecoregions. We begin by using analysis of covariance (ANCOVA) to test for significant differences among regions (ecoregions or bioregions) and identify significant interactions of nutrient concentration responses to human land uses among regions. We then used regression models to estimate natural background conditions under the condition of no human land cover.

### *5.1.1 Comparisons of land uses among different Ecoregions and Bioregions*

Agriculture is the dominant human land use in Mississippi; however, no significant patterns of different land uses among ecoregions or bioregions were observed (Figure 5.1). Although percent human land use (both % agricultural land use and % urban land use) varies among different ecoregions and bioregions, the median percent urban land use is relatively similar among ecoregions, except ecoregion 75. The first step in this analysis was to determine if significant interactions existed among bioregions and land use effects. We examined the interactions among bioregions and land uses using analysis of covariance (ANCOVA) and these interactions were found to have a significant impact on TN and TP concentrations. A previous study indicated strong interactions among land uses, ecoregions, and bioregions in models for both TN and TP (MDEQ 2003a, 2007a, 2009). Therefore, bioregion classification further reduced variation associated with natural geographic differences in nutrient concentrations due to geology, hydrology, and other factors.

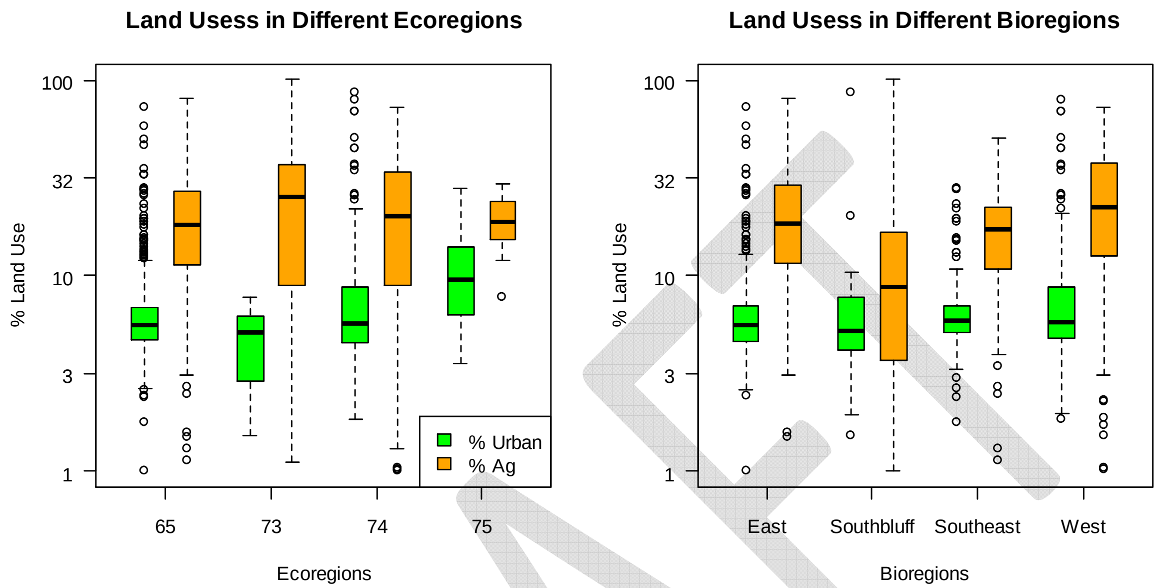


**Figure 5.1 - Boxplots of human land uses in different ecoregions and bioregions.**

The first step in this analysis was to determine if significant interactions existed among bioregions and land use effects. We examined the interactions among bioregions and land uses using analysis of covariance (ANCOVA) and these interactions were found to have a significant impact on TN and TP concentrations. A previous study indicated strong interactions among land uses, ecoregions, and bioregions in models for both TN and TP (MDEQ 2003a, 2007a, 2009). Therefore, bioregion classification further reduced variation associated with natural geographic differences in nutrient concentrations due to geology, hydrology, and other factors.

We further examined differences in nutrient concentrations among different ecoregions and bioregions (Figure 5.2). TN concentrations were relatively consistent across different ecoregions and bioregions in all stations, including reference stations, and TP concentrations were significantly different among both ecoregions and bioregions ($p<0.01$, ANCOVA). TP concentrations in the Southeast and East bioregions (or ecoregion 65) were significantly lower (ANCOVA $p<0.05$) than in the West and South Bluff bioregions (ecoregion 74) (Figure 5.2).

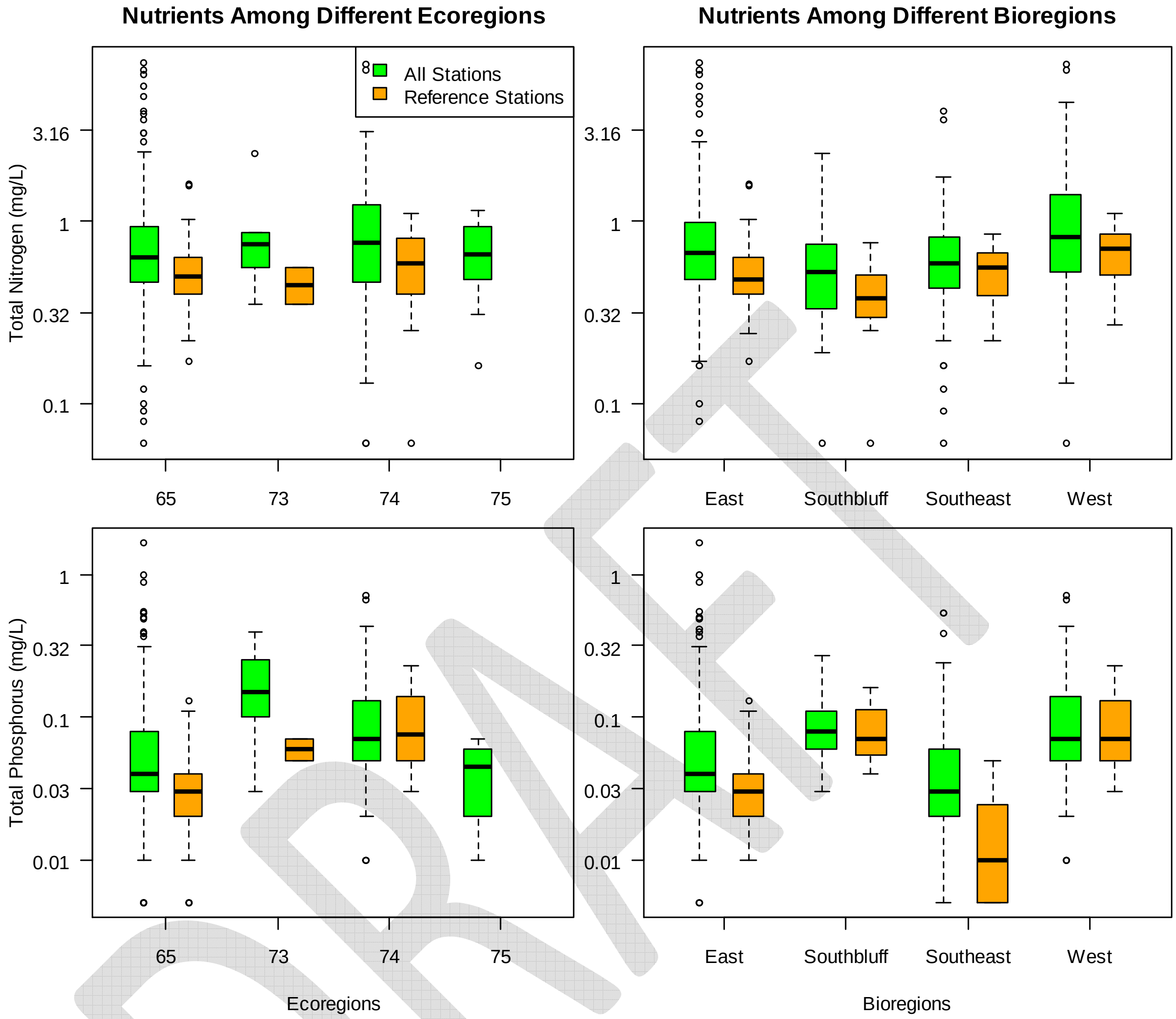




**Figure 5.2 Box plots of cropland, pasture and grassland, urban land, TP, TN, and NOx for M-BISQ sites in Mississippi in different bioregions. Center lines of boxes are the medians, tops and bottoms of boxes are 75th and 25th percentiles, respectively. Bars are 95% confidence intervals, and outliers are plotted as open points.**

### *5.1.2 Correlations between land use vs. nutrient concentrations*

Nutrient concentrations in streams may or may not be caused by increasing human disturbances in the watershed in all regions. To determine the association between nutrient concentrations and land uses, we first examined the correlations between different types of land uses in the watershed and different nutrient variables (Table 5.1). The strongest association was between TN and total human disturbances in the watershed. Total phosphorus concentrations in the watershed were less strongly associated with land uses probably for two reasons: first, phosphorus is more likely to be absorbed by soil before it reaches the stream channel in a well buffered riparian area; second, the precision of the phosphorus measurement (the majority of the TP concentrations were recorded at the same level) was low. Since Spearman correlation is calculated based on the rank of TP concentrations, many repeat ranks may affect the correlation coefficients.

**Table 5.1 Spearman correlation coefficients between nutrient concentrations and percent land use in the watershed.**

| % Land | TN | NOx | TP | Turbidity |
|---|---|---|---|---|
| Water | 0.296 | 0.251 | 0.257 | 0.2 |
| Wetland | 0.028 | -0.179 | -0.097 | -0.075 |
| Forest | **-0.502** | -0.399 | -0.304 | -0.173 |
| Natural | **-0.521** | **-0.514** | -0.355 | -0.248 |
| Urban | 0.352 | 0.367 | 0.256 | 0.043 |
| Cropland | 0.361 | 0.444 | 0.255 | 0.351 |
| PastGras | 0.445 | 0.399 | 0.317 | 0.178 |
| Agr | 0.49 | 0.49 | 0.353 | 0.315 |
| Human | **0.584** | **0.539** | 0.425 | 0.302 |

The figures below (Figure 5.3) demonstrate the combined effect of urban and agricultural land on nutrient concentrations.

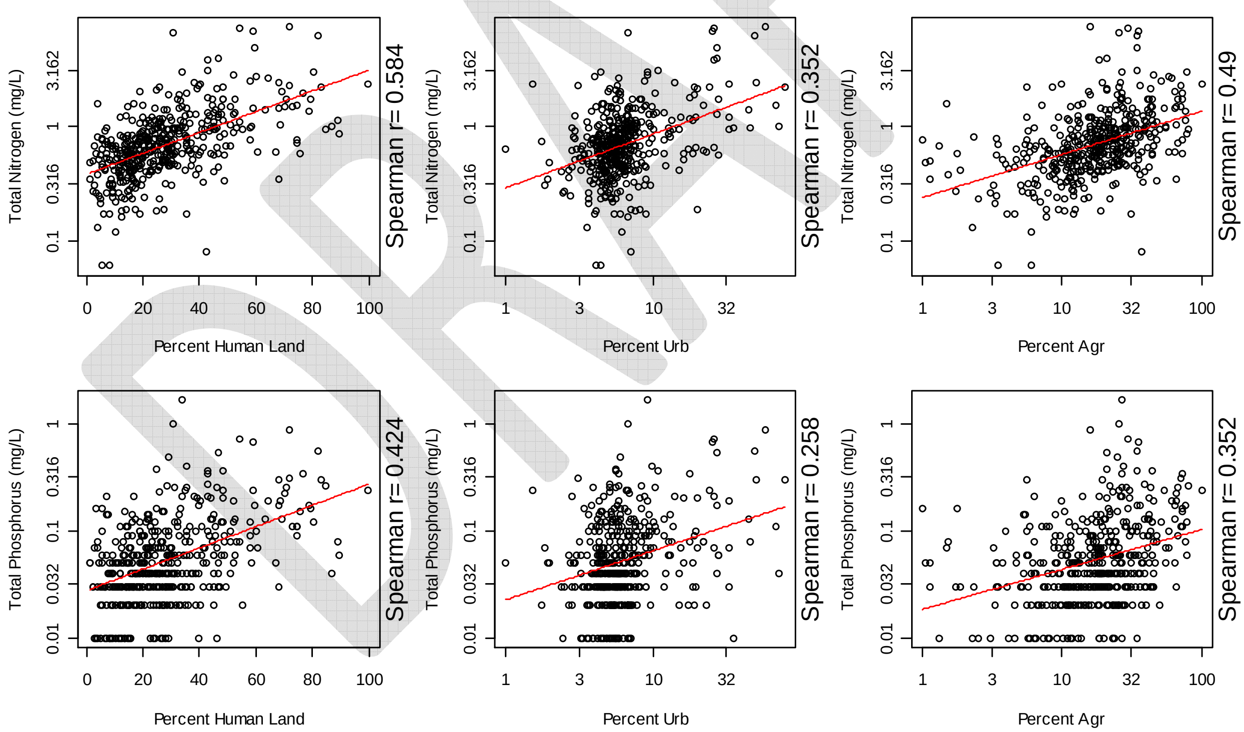


**Figure 5.3 Relationship between human land use and nutrient concentrations in the watershed.**

We developed predictive models using TN and TP concentrations as response variables and percent human land uses as predictors using the M-BISQ dataset. Although we expected that nutrient concentrations would most likely vary among ecoregions, we realize that macroinvertebrate assemblages were also expected to vary more strongly across bioregions. Since there were significant interactions between bioregions and land use classes, separate multiple regressions were performed for each individual bioregion. In the East bioregion, percent pasture and grassland and percent urban land contributed significantly to both TN and TP concentrations. The TN model for this region explained 37 percent of the total variance, which is better than the TP model (28.3%). The TN model in the West bioregion was stronger than in the East bioregion, explaining more than half of the total variance. Also, all three land use categories, including percent cropland, contributed to predicting nutrient concentrations in this bioregion. Both TN and TP models for the Southeast bioregion were weak ($R^2$ = 0.188 and 0.070); weak model results were probably due to short nutrient gradients in this region. The regression models for the South Bluff bioregion were significant but were based on relatively small sample sizes.

Using the regression models, the intercepts (constants) of the regressions were used to estimate those nutrient concentrations when human land uses were all equal to zero. The confidence intervals of the intercept were also calculated from the regression models (Table 5.2). According to this approach, the natural background TN concentration was approximately 0.193 mg/L in ecoregion 65 and 0.177 mg/L in ecoregion 74 (Figure 5.4). The natural TP concentration in ecoregion 65 (0.012 mg/L) was much lower than ecoregion 74 (0.030 mg/L). Due to a relatively small sample size, the estimated natural TP concentration in ecoregion 75 had a larger confidence interval (ranged from 0~0.021 mg/L, which covered the range of TP concentrations in ecoregion 65).



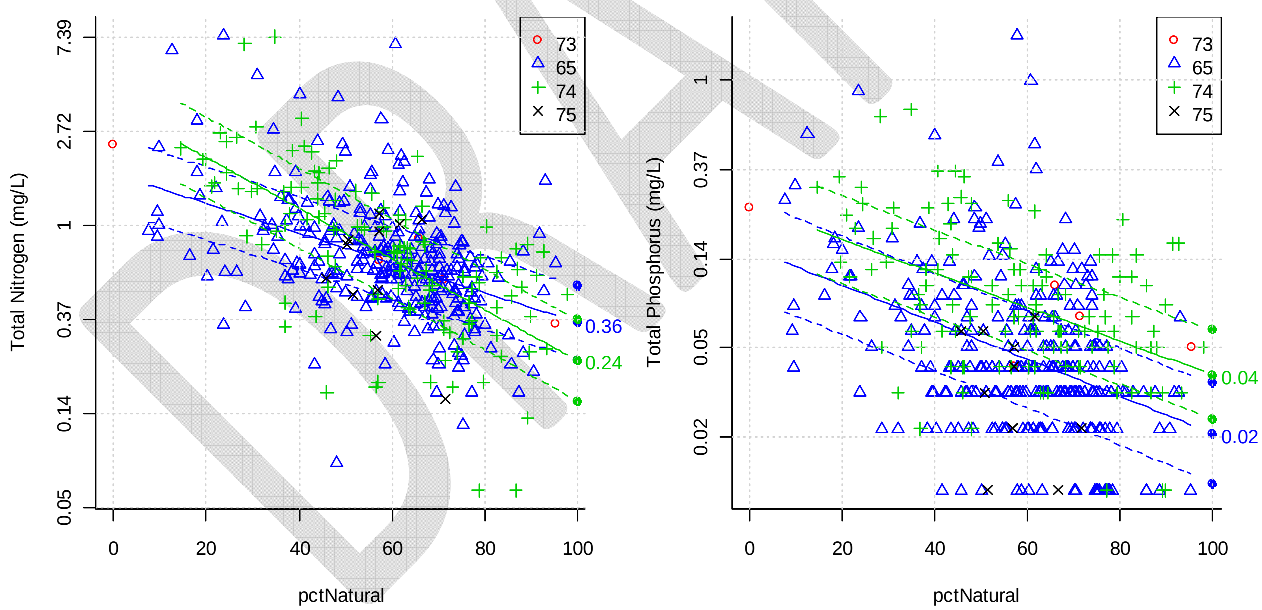


**Figure 5.4 Extrapolation of nutrient concentrations under natural conditions.**

We also ran these models by bioregion (Figure 5.5). The East and Southeast bioregions included the same regions as ecoregion 65 and 75. Both bioregion and ecoregion models had similar behavior, namely that TN and TP models for the Southeast bioregion were based on smaller sample sizes and explained less variance. As a result, it may have been more powerful to combine the entire region as one single region for use in extrapolation. Moreover, the extrapolated TN and TP concentrations for the East bioregion were very similar to the Southeast bioregion, indicating that the background nutrient concentrations could be similar in these two bioregions.

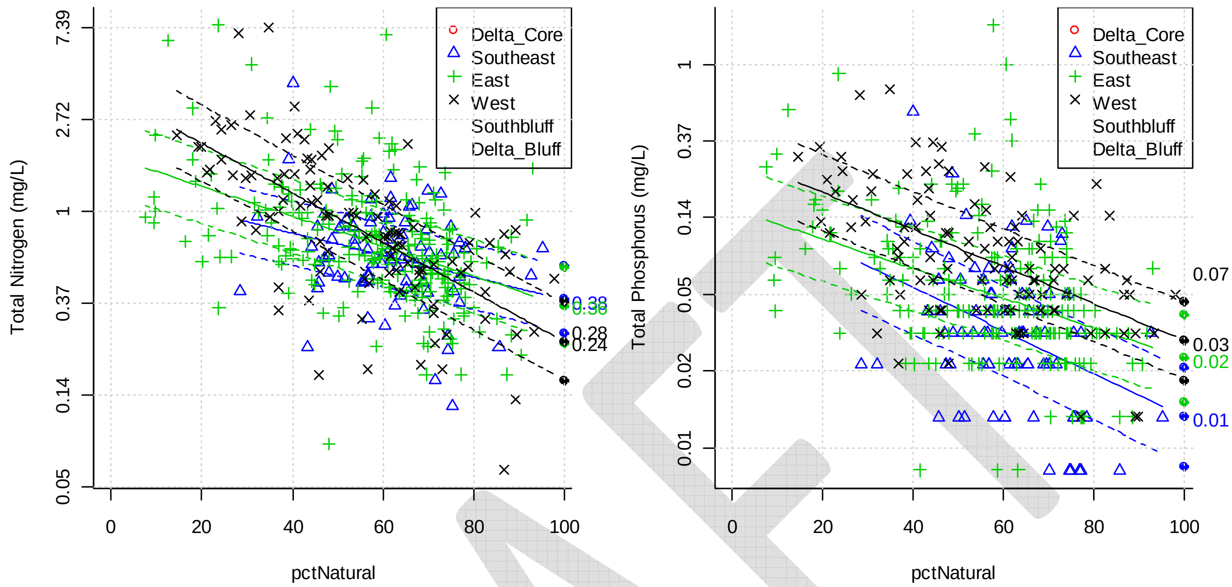


**Figure 5.5 Extrapolation of nutrient concentrations under natural conditions in each bioregion.**

**Table 5.2 Results of regression extrapolation from linear regression models.**

| Ecoregion | Nutrient Parameter | Mean | 50% prediction interval | 50% prediction interval |
|---|---|---|---|---|
| East | TN | 0.36 | 0.24 | 0.54 |
| | TP | 0.02 | 0.01 | 0.04 |
| Southeast | TN | 0.38 | 0.26 | 0.56 |
| | TP | 0.01 | 0.01 | 0.02 |
| South Bluff | TN | 0.28 | 0.17 | 0.45 |
| | TP | 0.07 | 0.05 | 0.1 |
| West | TN | 0.24 | 0.16 | 0.37 |
| | TP | 0.03 | 0.02 | 0.05 |
| 65 | TN | 0.36 | 0.24 | 0.53 |
| | TP | 0.02 | 0.01 | 0.03 |
| 74 | TN | 0.24 | 0.15 | 0.37 |
| | TP | 0.04 | 0.02 | 0.06 |

## 5.2 Thresholds from Least Disturbed Condition (LDC) reference site distributions

### *5.2.1 Criteria based on LDC 75th percentiles*

Conditions of least human disturbance are presumed to support designated uses due to the reduced levels of human induced stressors and, therefore, by definition represent an estimate of chemical and biological integrity consistent with the protection of those uses. The statewide dataset for M-BISQ development provided an ideal dataset to derive nutrient criteria using the LDC approach. Least disturbed stations were identified for the M-BISQ development process based on regional land use, stream physical habitat, and chemical characteristics. The M-BISQ recalibration refined the selection criteria for LDC streams in the state. For the purpose of nutrient criteria development, we used two different selection criteria for LDC in the original report (See Appendix D in MDEQ 2009 for analysis details). For this report, however, we use only the first of those, using the same criteria used as the MBISQ reference site selection process, to be most consistent with the M-BISQ development (Table 5.3). To avoid circularity, however, we removed nutrient parameters in the selection criteria, which only added one additional site to the original M-BISQ LDC site list.

**Table 5.3 Reference site selection criteria for LDC (Ag = agriculture, NPDES = distance to permitted discharge).**

| Ecogroup | %Natural | %Natural Buffer | Habitat Score | Chloride | NPDES |
|---|---|---|---|---|---|
| 1 or 2 | >50 | >60 | >100 | <10 | >5km |
| 3 | >70 | >80 | >110 | <10 | >5km |
| 4 | >70 | >80 | >110 | <10 | >5km |
| 5 | >70 | >80 | >110 | <30 | >5km |
| 6 | >70 | >80 | >100 | <30 | >5km |

The addition of sites sampled subsequent to the first report did not change the number of LDC sites appreciably over the original report (MDEQ 2009). Approximately 115 sites were identified as LDC and used. The LDC sites were also evenly distributed across the state than LDC2 since regional differences were incorporated into the selection process. However, there were still no LDC sites within ecoregion 75 (Southern Coastal Plain).

EPA's Technical Guidance Manual for Developing Nutrient Criteria for Streams and Rivers (USEPA, 2000) advocates selecting the 75$^{th}$ percentile of a distribution of reference condition values as a recommended target for a sufficiently protective value that provides an appropriate margin of safety. To estimate the 75$^{th}$ percentile of a distribution requires a relatively large sample size. From a biological survey standard point, a sample size of 30 is considered a minimum for estimating means and variances. Since percentile distribution is very sensitive to sample size, we ideally require at least 20 sites with one class to estimate a percentile for a distribution. Some of the bioregions (e.g., South Bluff), had far less than 20 (Table 5.3). As a result, although we develop nutrient benchmarks for each bioregion using the LDC approach, the sample size of LDC affects confidence in the representativeness of thresholds based on this approach for some regions. For example, the sample size of LDC for the South Bluff bioregion was only 9. Biological criteria for the West bioregion were derived separately for ecogroup 1 (northern west bioregion) and ecogroup 5 (southern west bioregion) because of differences in land use between these two ecogroups within that region. As a result, it was felt that separate nutrient benchmarks should be explored for these two ecogroups. Therefore, we examined percentile distributions of nutrient variables in each bioregion and ecoregion, and in addition, for ecogroups 1 and 5. But these ecogroups also had very small sample sizes (N=9). The distribution of LDC for TN and TP were summarized for different regions (Table 5.4).

**Table 5.4 LDC Percentile distribution and reference nutrient concentrations. Values in red are 75th percentiles.**

| | *Bioregion* | | | | *Ecoregion* | | *Ecogroup* | |
|---|---|---|---|---|---|---|---|---|
| | **East** | **South Bluff** | **West** | **Southeast** | **65** | **74** | **5** | **1** |
| **TN (mg/L)** | | | | | | | | |
| Min | 0.15 | 0.203 | 0.201 | 0.22 | 0.15 | 0.201246 | 0.201 | 0.431 |
| 25th | 0.366 | 0.255 | 0.423 | 0.370 | 0.367 | 0.380 | 0.379 | 0.632 |
| median | 0.485 | 0.380 | 0.685 | 0.422 | 0.480 | 0.461 | 0.420 | 0.745 |
| mean | 0.491 | 0.378 | 0.586 | 0.445 | 0.482 | 0.508 | 0.484 | 0.708 |
| **75th** | **0.635** | **0.461** | **0.782** | **0.579** | **0.630** | **0.760** | **0.736** | **0.834** |
| max | 1.590 | 0.760 | 1.198 | 0.850 | 1.590 | 1.198 | 0.880 | 1.198 |
| N | 71 | 9 | 18 | 17 | 88 | 25 | 9 | 9 |
| **TP (mg/L)** | | | | | | | | |
| Min | 0.005 | 0.05 | 0.007 | 0.005 | 0.005 | 0.007 | 0.007 | 0.06 |
| 25th | 0.022 | 0.060 | 0.052 | 0.005 | 0.020 | 0.060 | 0.030 | 0.089 |
| median | 0.033 | 0.080 | 0.077 | 0.020 | 0.030 | 0.080 | 0.050 | 0.108 |
| mean | 0.032 | 0.090 | 0.072 | 0.015 | 0.028 | 0.079 | 0.045 | 0.115 |
| **75th** | **0.047** | **0.126** | **0.111** | **0.030** | **0.042** | **0.126** | **0.071** | **0.151** |
| max | 0.130 | 0.180 | 0.275 | 0.050 | 0.130 | 0.275 | 0.140 | 0.275 |
| N | 71 | 9 | 18 | 17 | 88 | 25 | 9 | 9 |

Nutrient concentrations varied among different regions according to the LDC reference site distribution (Table 5.4). TN and TP LDC reference thresholds in East, Southeast, and South Bluff bioregions were very similar and lower than those in the West; again, the final threshold for the South Bluff bioregion warrants caution due to the small sample size.

### *5.2.2 Criteria based on 75$^{th}$ percentiles of BHC population*

Using biological criteria defined by M-BISQ scores for each bioregion (the lower quartile of M-BISQ07 reference site, Table 5.5), we identified 214 sites attaining the biological criterion. These sites (Appendix B) were used to define the BHC, and we derived nutrient benchmarks for different bioregions using this BHC population.

**Table 5.5 Selection criteria for BHC based on M-BISQ scores.**

| Bioregion | M-BISQ score |
|---|---|
| East | >65.7 |
| South Bluff | >55.9 |
| South East | >66 |
| West Bioregion - ecogroup 1 | >38.5 |
| West Bioregion - ecogroup 5 | >52.3 |

TN benchmarks estimated using BHC sites were mostly similar to each other among different bioregions, except ecogroup 1 which was higher than the other bioregions (Table 5.6). TP concentrations and turbidity varied more among regions. Generally, ecogroup 1 in the West bioregion and the South Bluff bioregions had higher TP concentrations than the other bioregions.

The percentiles estimated from BHC sites and nutrient benchmarks for each bioregion and ecoregion are listed in Table 5.6. The West bioregion was split into two ecogroups (ecogroup 1 in the North and ecogroup 5 in the South) because different biological criteria were developed for these two ecogroups. Using the 75$^{th}$ percentile of BHC, TN benchmarks were highest in the West bioregion (1.12 mg/L in ecogroup 1 and 0.770 mg/L in ecogroup 5). South Bluff bioregion had only 6 BHC sites, so it would be more appropriate to either adopt benchmarks from ecogroup 5 (adjacent neighbor) or combine the data into the whole ecoregion 74 (TN=0.0.843 mg/L). Similarly, TP benchmarks were highest in ecogroup 1 (0.120 mg/L) and lowest in the Southeast bioregion (0.040 mg/L). The TP benchmark in ecogroup 5 was 0.070 mg/L. The TP benchmark for the South Bluff bioregion would be 0.087 mg/L if it was combined into the whole ecoregion 74.

**Table 5.6 Percentile distribution of BHC nutrient concentrations.**

| | *Bioregion* | | | | *Ecoregion* | | | *Ecogroup* | |
|---|---|---|---|---|---|---|---|---|---|
| | ***East*** | ***South Bluff*** | ***West*** | ***Southeast*** | ***65*** | ***74*** | ***75*** | ***5*** | ***1*** |
| **TN (mg/L)** | | | | | | | | | |
| Min | 0.19 | 0.23 | 0.17 | 0.06 | 0.06 | 0.17 | 0.19 | 0.20 | 0.17 |
| 25th | 0.37 | 0.25 | 0.42 | 0.41 | 0.39 | 0.39 | 0.41 | 0.38 | 0.61 |
| median | 0.53 | 0.38 | 0.72 | 0.55 | 0.55 | 0.70 | 0.49 | 0.63 | 0.83 |
| mean | 0.53 | 0.41 | 0.66 | 0.50 | 0.52 | 0.63 | 0.45 | 0.55 | 0.81 |
| **75th** | **0.74** | **0.61** | **0.94** | **0.68** | **0.72** | **0.93** | **0.59** | **0.78** | **1.15** |
| max | 1.77 | 0.87 | 4.72 | 1.75 | 1.77 | 4.72 | 0.68 | 1.76 | 4.72 |
| N | 124 | 9 | 76 | 94 | 210 | 80 | 8 | 41 | 35 |
| **TP (mg/L)** | | | | | | | | | |
| Min | 0.01 | 0.05 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.03 |
| 25th | 0.03 | 0.05 | 0.04 | 0.02 | 0.02 | 0.04 | 0.01 | 0.03 | 0.06 |
| median | 0.03 | 0.08 | 0.06 | 0.03 | 0.03 | 0.06 | 0.02 | 0.04 | 0.10 |
| mean | 0.04 | 0.08 | 0.06 | 0.03 | 0.03 | 0.06 | 0.02 | 0.04 | 0.09 |
| **75th** | **0.05** | **0.11** | **0.10** | **0.05** | **0.05** | **0.10** | **0.05** | **0.06** | **0.13** |
| max | 0.40 | 0.16 | 0.51 | 0.39 | 0.40 | 0.51 | 0.06 | 0.15 | 0.51 |
| N | 124 | 9 | 76 | 94 | 210 | 80 | 8 | 41 | 35 |

## 5.3 Within site variability

A number of approaches exist to evaluate within site variability and characterize uncertainty around the proposed thresholds using the LDC and BHC distribution based methods. Since many of the sites (about half of the entire population within the State) have been sampled more than once, within site variability could be used to estimate uncertainty associated with the above thresholds. We resampling from the distributions using a bootstrapping approach based on within site variability estimates from the replicate sampling data to estimate this uncertainty and place confidence intervals around the distribution estimates (Table 5.7).

**Table 5.7 Nutrient benchmarks and their confidence intervals using distribution based approach**

| | | ***Bioregion*** | | | | ***Ecoregion*** | | | ***Ecogroup*** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ***East*** | ***South Bluff*** | ***West*** | ***Southeast*** | ***65*** | ***74*** | ***75*** | ***5*** | ***1*** |
| **LDC sites - 75th percentile and 95% confidence intervals** | | | | | | | | | | |
| **TN** | **Median** | 0.64 | 0.52 | 0.84 | 0.64 | 0.64 | 0.78 | | 0.78 | 0.92 |
| | **5% CI** | 0.60 | 0.38 | 0.78 | 0.54 | 0.61 | 0.71 | | 0.53 | 0.81 |
| | **95% CI** | 0.70 | 0.72 | 0.93 | 0.71 | 0.70 | 0.84 | | 0.88 | 1.07 |
| **TP** | **Median** | 0.05 | 0.12 | 0.12 | 0.03 | 0.04 | 0.14 | | 0.07 | 0.14 |
| | **5% CI** | 0.04 | 0.08 | 0.09 | 0.02 | 0.04 | 0.10 | | 0.05 | 0.10 |
| | **95% CI** | 0.05 | 0.16 | 0.14 | 0.04 | 0.05 | 0.16 | | 0.14 | 0.21 |
| **Biological reference sites - 75th percentile and 95% confidence intervals** | | | | | | | | | | |
| **TN** | **Median** | 0.76 | 0.61 | 1.02 | 0.71 | 0.74 | 0.95 | 0.56 | 0.80 | 1.17 |
| | **5% CI** | 0.68 | 0.45 | 0.94 | 0.67 | 0.68 | 0.88 | 0.48 | 0.76 | 1.02 |
| | **95% CI** | 0.80 | 0.76 | 1.11 | 0.81 | 0.77 | 1.02 | 0.68 | 0.88 | 1.22 |
| **TP** | **Median** | 0.05 | 0.10 | 0.10 | 0.05 | 0.05 | 0.10 | 0.05 | 0.07 | 0.14 |
| | **5% CI** | 0.04 | 0.08 | 0.08 | 0.04 | 0.05 | 0.08 | 0.02 | 0.06 | 0.11 |
| | **95% CI** | 0.06 | 0.16 | 0.12 | 0.05 | 0.06 | 0.11 | 0.06 | 0.07 | 0.18 |

## 5.4 Summary of nutrient benchmarks based on reference approaches

Nutrient benchmarks derived from different reference approaches varied across different bioregions (Table 5.8). Generally speaking, nutrient benchmarks derived using the natural background empirical modeling approach were lower than those using LDC and BHC conditions.

**Table 5.8 Summary of nutrient benchmarks from distribution based approaches. Confidence intervals are shown in parentheses. LDC = least disturbed reference condition, BHC = biologically healthy condition**

| | ***Bioregion*** | | | | ***Ecoregion*** | | | ***Ecogroup*** | |
|---|---|---|---|---|---|---|---|---|---|
| **Approach** | ***East*** | ***South Bluff*** | ***West*** | ***Southeast*** | ***65*** | ***74*** | ***75*** | ***5*** | ***1*** |
| | | | | **TN concentrations (mg/L)** | | | | | |
| **Natural Background** | 0.36 (0.24-0.54) | 0.38 (0.17-0.45) | 0.24 (0.16-0.37) | 0.38 (0.26-0.56) | 0.36 (0.24-0.53) | 0.24 (0.15-0.37) | | | |
| **LDC** | 0.64 (0.60-0.70) | 0.46 (0.38-0.72) | 0.78 (0.78-0.93) | 0.58 (0.54-0.71) | 0.63 (0.61-0.7) | 0.76 (0.71-0.84) | | 0.74 (0.53-0.88) | 0.83 (0.81-1.07) |
| **BHC** | 0.74 (0.68-0.80) | 0.61 (0.45-0.76) | 0.94 (0.94-1.11) | 0.68 (0.67-0.81) | 0.72 (0.68-0.77) | 0.93 (0.88-1.02) | 0.56 (0.48-0.68) | 0.78 (0.76-0.88) | 1.15 (1.02-1.22) |
| | | | | **TP concentrations (mg/L)** | | | | | |
| **Natural Background** | 0.02 (0.01-0.04) | 0.07 (0.05-0.1) | 0.03 (0.02-0.05) | 0.01 (0.01-0.02) | 0.02 (0.01-0.03) | 0.04 (0.02-0.04) | | | |
| **LDC** | 0.05 (0.04-0.05) | 0.13 (0.08-0.16) | 0.11 (0.09-0.14) | 0.03 (0.02-0.04) | 0.04 (0.04-0.05) | 0.13 (0.10-0.16) | | 0.07 (0.05-0.14) | 0.15 (0.10-0.21) |
| **BHC** | 0.05 (0.04-0.06) | 0.11 (0.08-0.16) | 0.10 (0.08-0.12) | 0.05 (0.04-0.05) | 0.05 (0.05-0.06) | 0.10 (0.08-0.11) | 0.05 (0.02-0.06) | 0.06 (0.06-0.07) | 0.13 (0.11-0.18) |

# 6 Causal relationship between nutrients and biological response

In the absence of a direct linkage between nutrient concentrations and direct response variables (e.g., algal biomass and species compositions), indirect response variables, such as macroinvertebrate metrics, were used to relate possible biological response to nutrient concentrations. Biological degradation, indicated by the decline of macroinvertebrate compositional metric values, may or may not correlate with nutrient enrichment since macroinvertebrate – nutrient relationships are and potentially confounded by other stressors. At the same time, other environmental stressors correlated with nutrient variables may confound the relationship between nutrients and biological response.

We used the propensity score approach to support the proposed causal linkage between nutrient pollution and biological response (USEPA 2010). The propensity score approach accounts for background effects of multiple co-varying stressors with nutrients and allows a user to then evaluate the effects of total phosphorus independent of these other stressors. The approach depends on identification of a number of streams with similar covariate distributions (other observed environmental factors), but which differ in nutrient concentrations (USEPA 2010). In the case of only a single factor (e.g., sediment) covarying with nutrient concentrations, we could simply stratify the data set by this single factor (i.e., identify bins of sites with similar sediment concentrations), but there are frequently many factors that co-vary with nutrients and we need to control for all of them.

Propensity functions (Imai and Van Dyk, 2004 and Yuan 2010) summarize the contributions of all known covariates as a single parameter. A propensity function is defined as the conditional probability of a multivariate treatment (e.g., different nutrient concentrations), given values of known covariates. This conditional probability can be characterized by a single parameter, referred to here as the propensity score, which is the mean expected value of the treatment. For example, observed nutrient concentrations can be modeled as a function of covariate values using regression analysis, and the predicted mean nutrient concentration in each stream is the propensity score (USEPA 2010). Then, stratifying or binning by propensity scores effectively splits the data set into groups with similar covariate distributions. Once the data set is stratified, causal effects of nutrients can be more confidently estimated within each group because distributions of other covariates are similar (Yuan 2010). While effect thresholds can be identified, they would not be feasible to apply because of uncertainty in assigning new sites to propensity score classes.

The specific steps in the propensity score analysis include 1) identifying a suite of environmental variables that co-vary with nutrient concentrations, 2) using an empirical model like generalized linear models or multiple regression models (with appropriately transformed values) to summarize the covariates and predict nutrient concentrations, 3) stratifying the predicted TP (propensity scores) into different bins, corresponding to perceived changes in TP expectations along the propensity score axis, and 4) characterizing relationships between biological responses and nutrient concentration in each of the strata.

## 6.1 Correlations among environmental variables

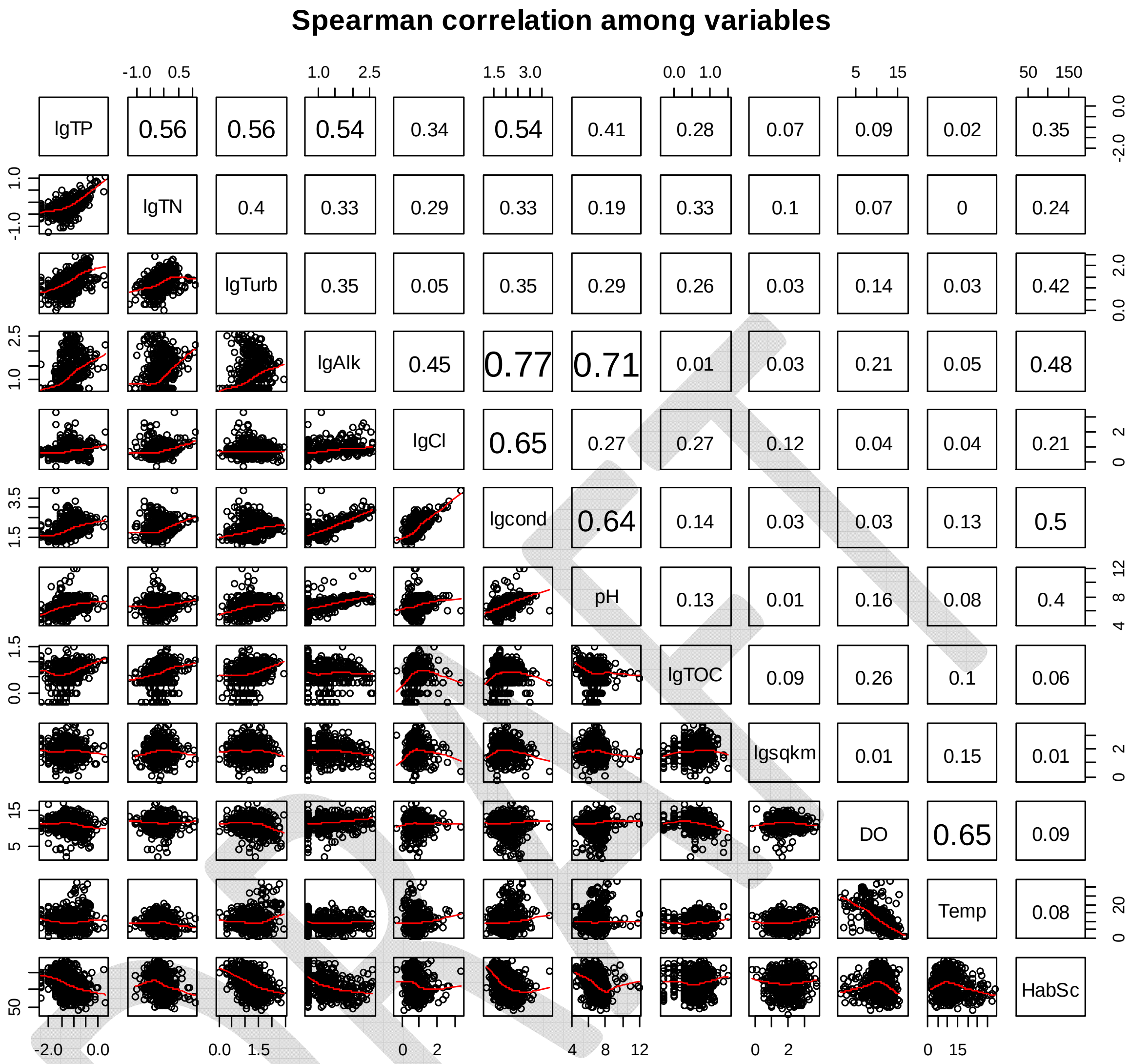


**Figure 6.1 Spearman correlation among environmental variables.**

We first examined correlations among environmental parameters that might be correlated with nutrient parameters and may also potentially contribute to biological degradation (Figure 6.1). We were particularly interested in nutrient related parameters, such as dissolved oxygen (DO) concentrations and turbidity since these variables are directly linked to aquatic life uses in streams. We did not find significant relations between DO and other chemical parameters but found strong correlations between turbidity and both TN and TP concentrations. Another common stressor, specific conductance, was strongly correlated with Cl concentrations, pH, alkalinity, and TP.

## 6.2 Correlations of macroinvertebrate metrics with nutrients

We used data collected from the M-BISQ program to examine relationships between macroinvertebrate indices and metrics and nutrient parameters. Correlation analysis identifies apparent linkages between biological condition and environmental variables (Figure 6.2). It may or may not indicate the real relationship between biological condition (biological indices) and environmental characteristics. A number of nutrient related environmental variables were strongly correlated with M-BISQ scores and composite metrics in each bioregion (Table 6.1). Overall, M-BISQ scores were strongly correlated with conductivity, nutrients, alkalinity, chloride, turbidity, pH, and habitat variables ($p<0.05$) in most bioregions except the South Bluff ecoregion. TN concentration was a better predictor of the macroinvertebrate index than $NO_{2+3}$ concentration, reinforcing the applicability of TN criteria for nitrogen pollution.

**Table 6.1 Spearman correlation between macroinvertebrate metrics and environmental variables**

| | MBISQ | ntaxa_Ephem | ntaxa_EPT | %_EPTsens | ntaxa_toler | ntaxa_intol | ntaxa_total |
|---|---|---|---|---|---|---|---|
| lgcond | **-0.632** | -0.304 | **-0.482** | **-0.456** | **0.538** | **-0.593** | -0.377 |
| lgTP | **-0.501** | -0.246 | -0.398 | **-0.425** | **0.409** | **-0.45** | -0.198 |
| lgTN | **-0.456** | -0.271 | **-0.421** | **-0.449** | 0.359 | **-0.434** | -0.232 |
| lgAlk | **-0.635** | -0.279 | **-0.448** | **-0.461** | **0.521** | **-0.607** | -0.367 |
| lgCl | **-0.424** | -0.22 | -0.362 | -0.385 | 0.231 | -0.39 | -0.31 |
| lgTOC | -0.173 | -0.321 | -0.337 | -0.357 | 0.078 | -0.158 | -0.161 |
| lgTurb | **-0.485** | -0.251 | -0.366 | -0.303 | **0.496** | **-0.455** | -0.17 |
| lgsqkm | 0.146 | 0.305 | 0.277 | 0.19 | -0.119 | 0.196 | 0.16 |
| lgAmmonia | -0.249 | -0.142 | -0.234 | -0.184 | 0.287 | -0.203 | -0.047 |
| DOPct | -0.042 | 0.159 | 0.129 | 0.085 | 0.072 | -0.014 | -0.001 |
| DO | -0.074 | 0.066 | 0.043 | 0.051 | 0.16 | -0.049 | 0.002 |
| pH | **-0.461** | -0.081 | -0.226 | -0.269 | **0.408** | **-0.419** | -0.227 |
| DepthFeet | 0.141 | 0.071 | 0.097 | 0.057 | -0.095 | 0.148 | 0.107 |
| Flow | 0.292 | 0.358 | 0.385 | 0.26 | -0.248 | 0.307 | 0.235 |
| Temp | 0.108 | 0.101 | 0.106 | 0.034 | -0.196 | 0.101 | 0.018 |
| TotHabScore | **0.519** | 0.246 | 0.37 | 0.313 | **-0.476** | **0.557** | 0.311 |

## 6.3 Propensity scores

The environmental variables included in the propensity score analysis included conductivity, water temperature, alkalinity, chloride, hardness, and total suspended solids because they are also potentially associated with biological degradation in the State. Because total nitrogen is highly correlated with total phosphorus (Spearman rho - 0.80), effects observed on phosphorus after accounting for nitrogen are expected to be similar to effects of nitrogen after accounting for phosphorus. Therefore only effects with total phosphorus were examined and similar effects of nitrogen were then implied. A generalized linear model predicting TP using the co-variate predictors above was conducted with all data statewide, not by ecoregion. Using the six variables and a generalized linear model on log transformed data (except temperature), the predicted TP values (propensity scores) were stratified into four different classes, corresponding to perceived changes in TP expectations along the propensity score axis (Figure 6.3).

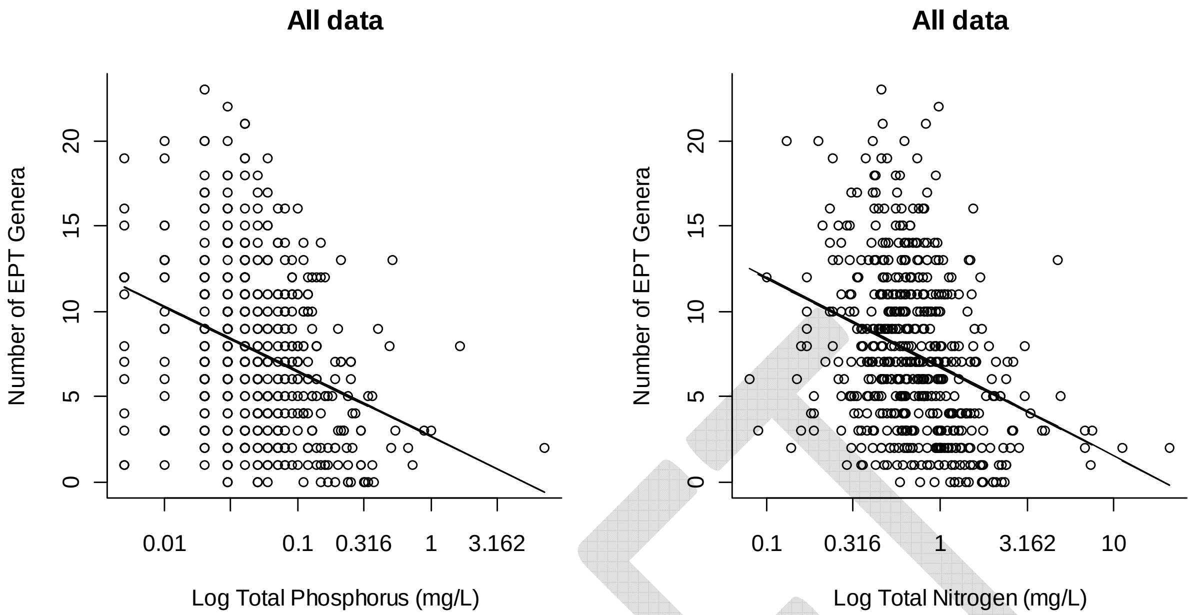


**Figure 6.2 Relationships between TP and TN concentrations and Number of EPT taxa.**

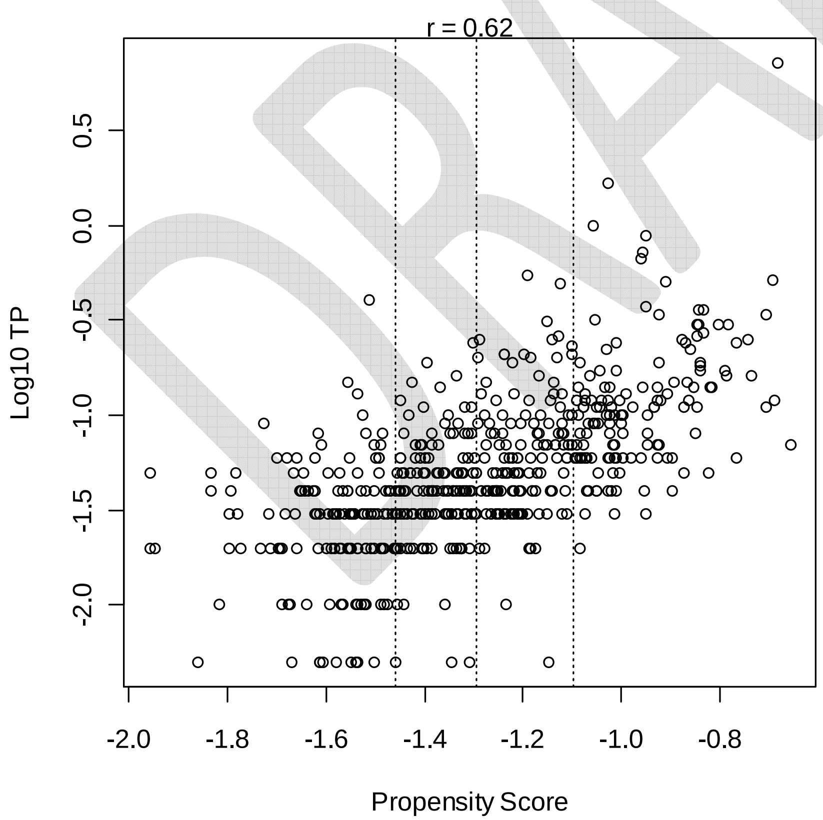


**Figure 6.3 Propensity Scores and observed TP concentrations. Vertical lines indicate equal-sample size bin boundaries.**

**Table 6.2 Correlations between TP and environmental covariables before and after stratification**

| TP | All | Stratum1 | Stratum2 | Stratum3 | Stratum4 |
|---|---|---|---|---|---|
| Lgcond | 0.39 | 0.14 | 0.17 | 0.14 | -0.19 |
| lgAlk | 0.45 | 0.12 | 0.08 | 0.03 | -0.02 |
| lgCl | 0.27 | 0.05 | 0.29 | 0.03 | -0.06 |
| pH | 0.26 | 0.16 | -0.01 | -0.01 | -0.02 |
| lgTurb | 0.49 | 0.00 | -0.10 | 0.11 | 0.31 |
| TotHabScore | -0.30 | 0.09 | -0.06 | -0.04 | -0.09 |
| Long_Dec | -0.23 | -0.13 | -0.31 | -0.02 | -0.10 |
| MBISQ | -0.45 | -0.13 | -0.01 | -0.15 | -0.23 |
| nt_EPT | -0.32 | 0.04 | -0.07 | -0.15 | -0.24 |

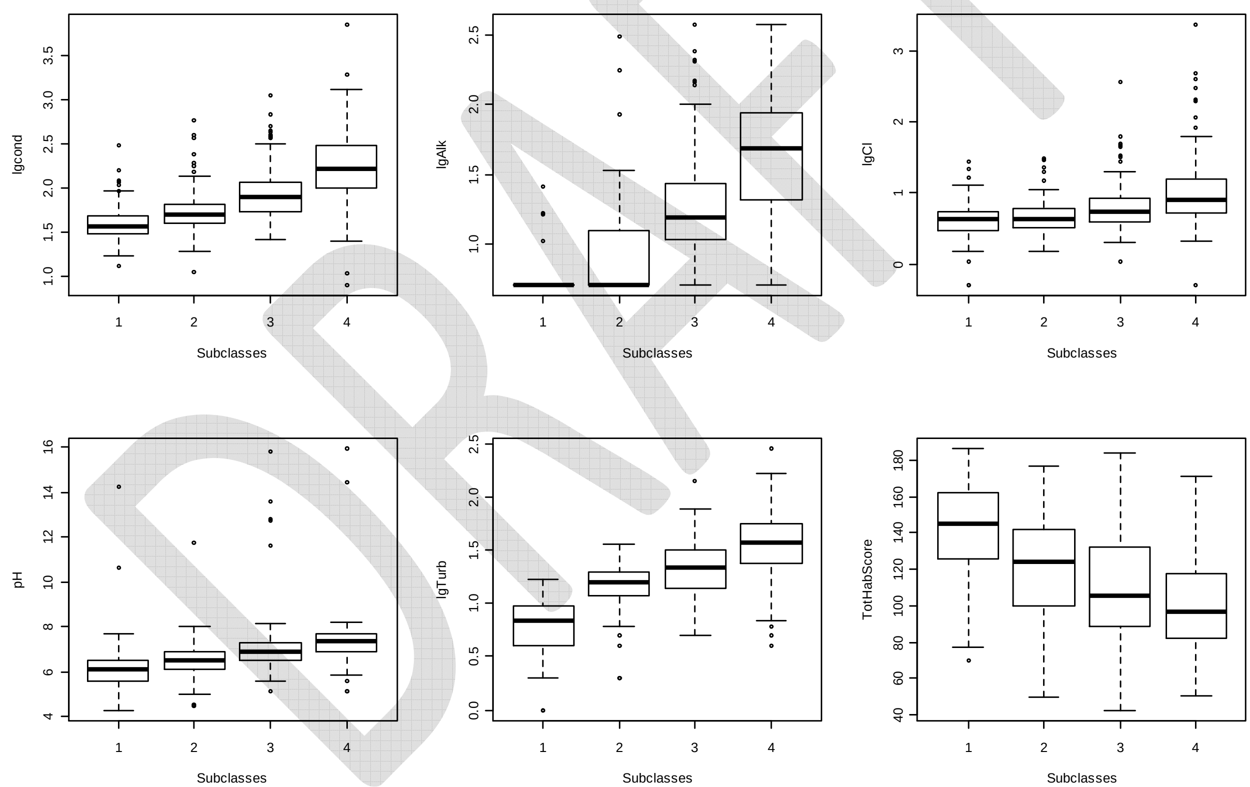


**Figure 6.4 Covariables in the four propensity subclasses. Clockwise from upper left: conductivity, alkalinity, chloride, pH, turbidity, and habitat.**

Correlations of the variables in the model with the propensity scores (Table 6.2) indicate that Class 1 (left of the first vertical line in Figure 6.3) has the lowest TSS and TKN as well as lower conductivity, alkalinity, temperature, chloride, and hardness, and somewhat higher chlorophyll *a* and dissolved oxygen. Sites with greater degrees of stress are in Class 4 (farthest right in the figure). Correlations of TP with covariates were greatly reduced within the classes in comparison to correlations in all sites (pooled classes). Correlations of TP with TN and TKN remained relatively high in the individual classes.

After propensity score adjustment, biological conditions including EPT taxa and other metrics remained correlated with nutrients especially in the third and fourth strata (Table 6.3 and Figure 6.5). These results support the causal hypothesis of nutrient effects on invertebrate responses even in the presence of covarying stressors and justify endpoints generated with the stressor-response analyses described in the next chapter.

**Table 6.3 Correlations between environmental variables and EPT taxa scores before and after data were stratified.**

| | Before | After | | | |
|---|---|---|---|---|---|
| EPT taxa | | Stratum1 | Stratum2 | Stratum3 | Stratum4 |
| lgcond | -0.39 | -0.07 | -0.14 | -0.34 | -0.14 |
| lgTP | -0.32 | 0.04 | -0.07 | -0.15 | -0.24 |
| lgTN | -0.32 | 0.01 | -0.20 | -0.26 | -0.31 |
| lgAlk | -0.37 | -0.03 | -0.07 | -0.12 | -0.14 |
| lgCl | -0.24 | 0.00 | -0.17 | -0.14 | -0.08 |
| pH | -0.11 | 0.16 | 0.23 | -0.06 | 0.03 |
| lgTurb | -0.35 | -0.14 | 0.03 | -0.04 | -0.03 |
| TotHabScore | 0.35 | 0.25 | 0.10 | 0.20 | 0.21 |
| Long_Dec | 0.04 | 0.03 | -0.04 | -0.17 | -0.09 |

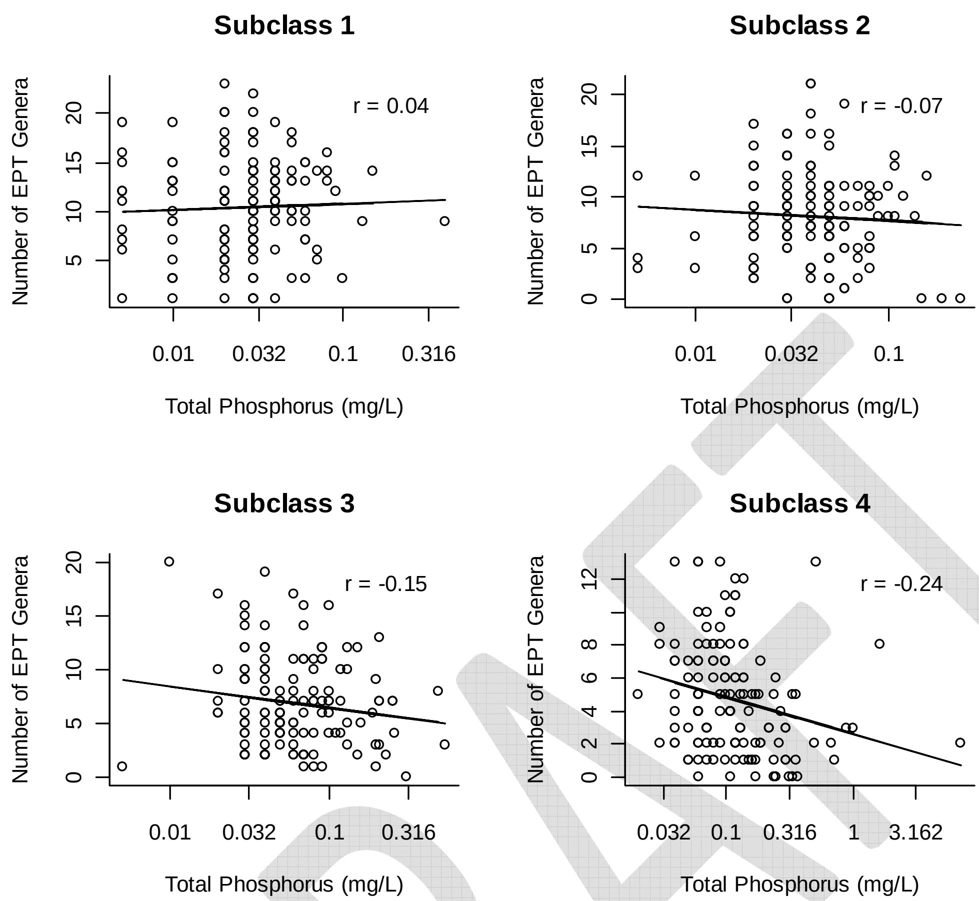


**Figure 6.5 Correlations of EPT taxa and TP concentrations in different propensity subclasses.**

# 7 Stressor- response approach

The propensity score analysis found only correlations between macroinvertebrate metrics and nutrient concentrations, supporting the causal models. However, after correcting for covariates, the strength of the correlations was reduced, therefore more caution is warranted when examining the macroinvertebrate indices and metrics relationships with nutrient parameters. We first used visual plots to further explore the relationships. We used either linear regression or a locally weighted average regression line to examine trends along environmental gradients. Due to regional differences, we examined the relationships for each bioregion. We then used a risk assessment approach employing logistic regression analysis to examine the risk of biological communities along increasing nutrient concentrations. We also employed change point analysis using nonparametric deviance reduction to identify ecological thresholds in the nutrient-invertebrate response relationships (Qian et al., 2003).

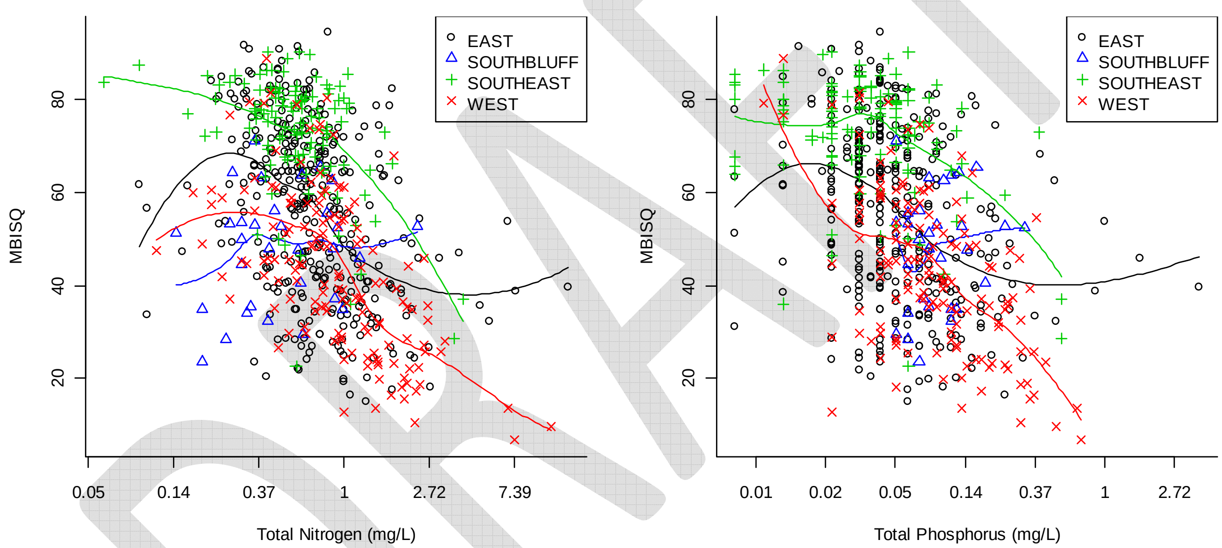


**Figure 7.1 Relationships between MBISQ scores and nutrient concentrations in different bioregions in Mississippi. Lines are LOWESS non-linear regressions for each region.**

The East bioregion had the largest sample size (375 sites) and exhibited biological responses to nutrient gradients (Figure 7.1). M-BISQ scores for the East bioregion declined with increased TN ($R^2$=0.15, p<0.001) and TP concentrations ($R^2$=0.12, p < 0.001); these relationships could be nonlinear since the linear models only explained a small amount of variance. According to the LOWESS regression lines (Figure 7.1), when TN approached 0.60 mg/L and TP approached 0.040 mg/L, M-BISQ scores declined sharply. M-BISQ scores also declined linearly along turbidity gradients.

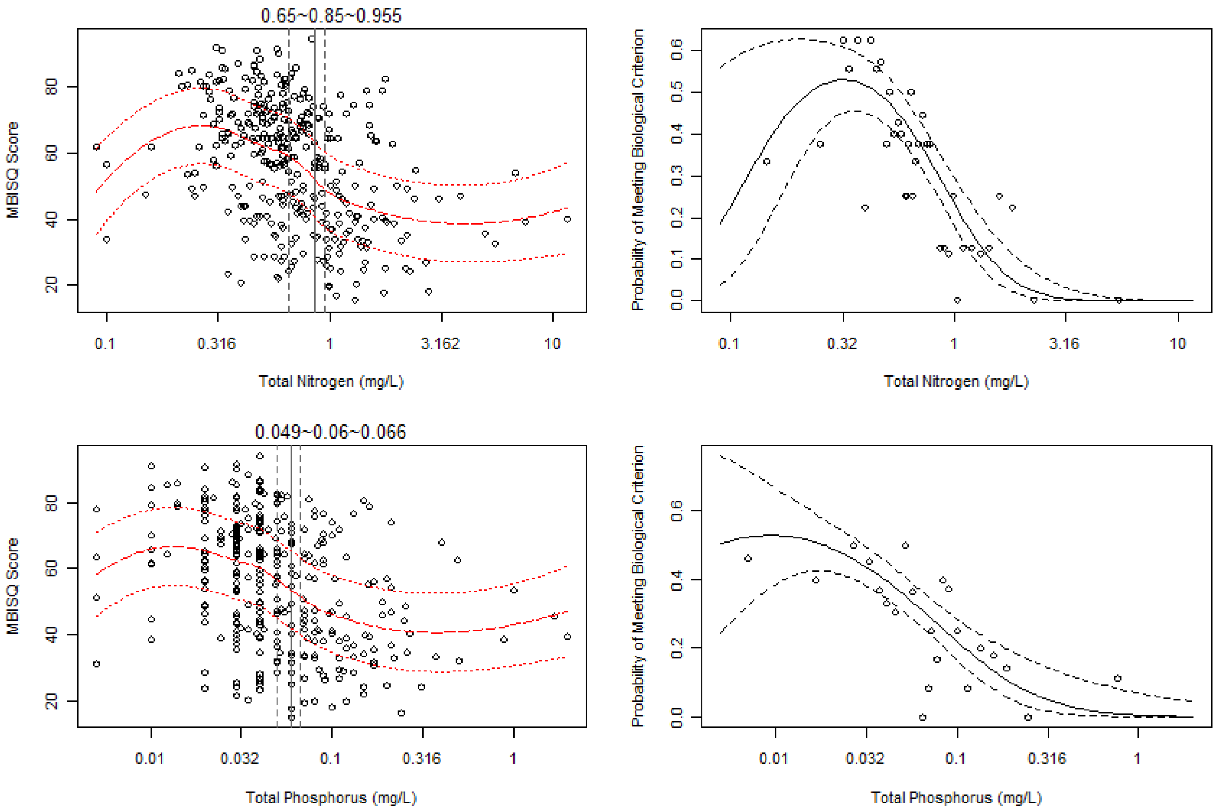


**Figure 7.2 Responses of M-BISQ score to nutrient parameters in East Bioregion. Raw data are plotted on the left and logistic probability curves on the right.**

Since the linear relationship only explained a small proportion of variance and indicated non-linear changes in responses to nutrients, we used nonparametric deviance reduction (change point analysis) to identify ecological response thresholds (Qian et al., 2003). This technique is based on regression tree models, which are used to predict the value of a continuous variable from one or more continuous variables. The change point in this application is the first split of a tree model when there is only a single predictor variable. When the split in the data minimizes the deviance, a threshold is identified. This approach has been used to detect ecological changes along environmental gradients (Qian *et al.*, 2003). Uncertainty in the deviance reduction change point (95 percent CIs) was estimated from empirical percentiles of a bootstrap distribution from resampling 1,000 times. We also used a logistic regression model to explore the risk of biological impairment with elevated nutrient concentrations. The logistic regression model used the biological condition benchmark for each region as the criterion to model the probability of the biological condition at a site being below the biological criteria along the nutrient gradients.

In the East bioregion (Figure 7.2), the tree based models explained more variance than linear models (better $R^2$ 0.19 vs. 0.15); from this, we concluded that the tree based model was better than the linear model. The change points were 0.85 mg/L TN and 0.06 mg/L TP. The logistic regression also showed similar decline with increasing nutrient concentrations (Figure 7.2).

Macroinvertebrate responses to nutrient gradients in the West bioregion showed a strong linear decline (Figure 7.3). The northern part of the West bioregion (ecogroup 1) was dominated by agricultural land uses while the southern part (ecogroup 5) was characterized by natural land use. M-BISQ scores in the southern West region were generally higher than in the northern West region (Figure 7.3). M-BISQ threshold criteria are 38.5 for the northern and 52.3 for the southern part of the bioregion. Similar to the East bioregion, macroinvertebrate M-BISQ scores declined with increased nutrient concentrations but with a much stronger linear decline (linear models, $R^2$=0.36 for TN 0.31 for TP, $p<0.001$). The strong linear decline enabled us to assume a linear decline of biological decline along the nutrient gradients in the East bioregion. Therefore, we applied a linear interpolation approach as recommended in USEPA (2010) from these models to determine thresholds based on biological criteria for these two ecogroups. Thresholds were 0.468 (0.242-0.904) with 50% confidence limits) mg/L TN and 0.038 (0.016-0.091) mg/L TP for the southern West bioregion (ecogroup 5), and 1.227 (0.635-2.369) mg/L TN and 0.123 (0.051-0.295) mg/L TP for the northern West bioregion (ecogroup 1).

As pointed out earlier, since different biological criteria were defined for the northern West (ecogroup 1) and southern West bioregions (ecogroup 5), we performed logistic regression analyses on two separate ecogroups (impairment threshold: M-BISQ score <38.5 for ecogroup 1 and <52.3 for ecogroup 5) (Figure 7.3). When the West bioregion was treated as a whole nutrient region, the thresholds were around 0.800 mg/L TN and 0.060 mg/L TP. When ecogroup 1 and ecogroup 5 were analyzed separately, these two regions had different nutrient thresholds. The nutrient thresholds were approximately 1 mg/L TN and 0.1 mg/L TP in ecogroup 1, compared to 0.8 mg/L TN and 0.07 mg/L TP in ecogroup 5.

Regression models for M-BISQ scores and TN and TP gradients in the ecogroups within the regions were much weaker compared to the whole region models. TN and TP models in ecogroup 1 (83 sites) were significant but explained little variance (TN: $R^2 = 0.242$, $p<0.001$, TP: $R^2 = 0.083$, $p=0.008$). TN models in ecogroup 5 (58 sites) were not significant (TN: $R^2 = 0.059$, $p=0.066$) and the TP model was also weak (TP: $R^2 = 0.264$, $p<0.001$).

The South Bluff and West bioregion belong to the same ecoregion (ecoregion 74). However, the South Bluff bioregion had a relatively small sample size for gradient analysis. As a result, in the South Bluff region, correlation analyses exhibited no observable responses of macroinvertebrate metrics (M-BISQ scores and metrics) to either TN or TP gradients ($p>0.05$).

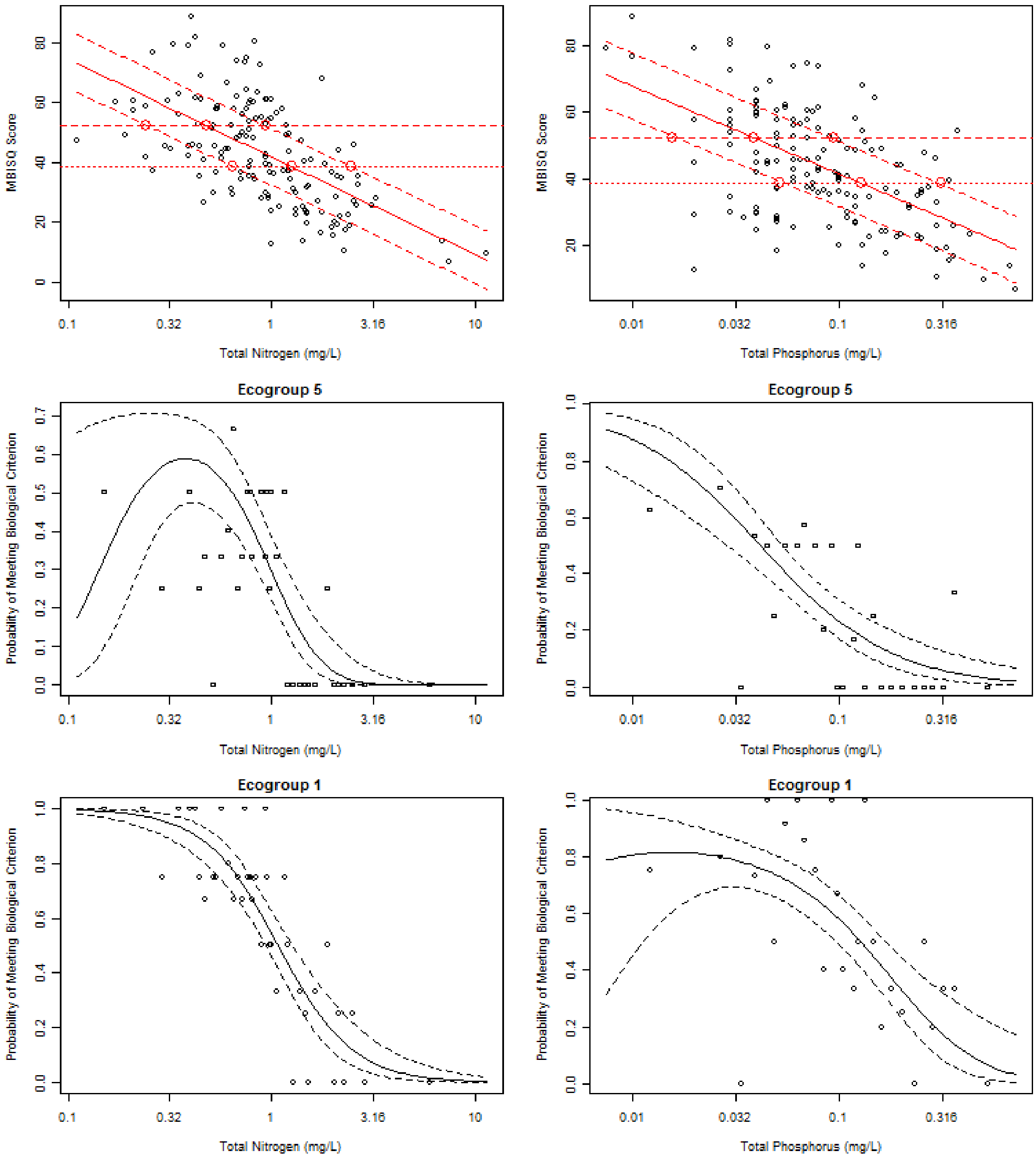


**Figure 7.3 Responses of M-BISQ scores to nutrient parameters in West Bioregion. The top two curves are raw data and the lower 4 curves are logistic probabilities. The horizontal red dashed lines are M-BISQ criteria for ecogroup 1 (lower line) and ecogroup 5 (upper line), respectively.**

Macroinvertebrate responses to nutrient gradients in the Southeast bioregion also showed a decline, but not in a linear fashion. Both linear and quadratic models were fit to the dataset and quadratic models fit the data significantly better than linear models (ANOVA p = 0.001). We used a piecewise regression technique to find the point of change in slope along the quadratic curve. The break points were at 0.31 mg/L (90% confidence limits 0.135 - 0.73) for TN and 0.03 7mg/L (90% confidence limits around 0.023 - 0.06) for TP.

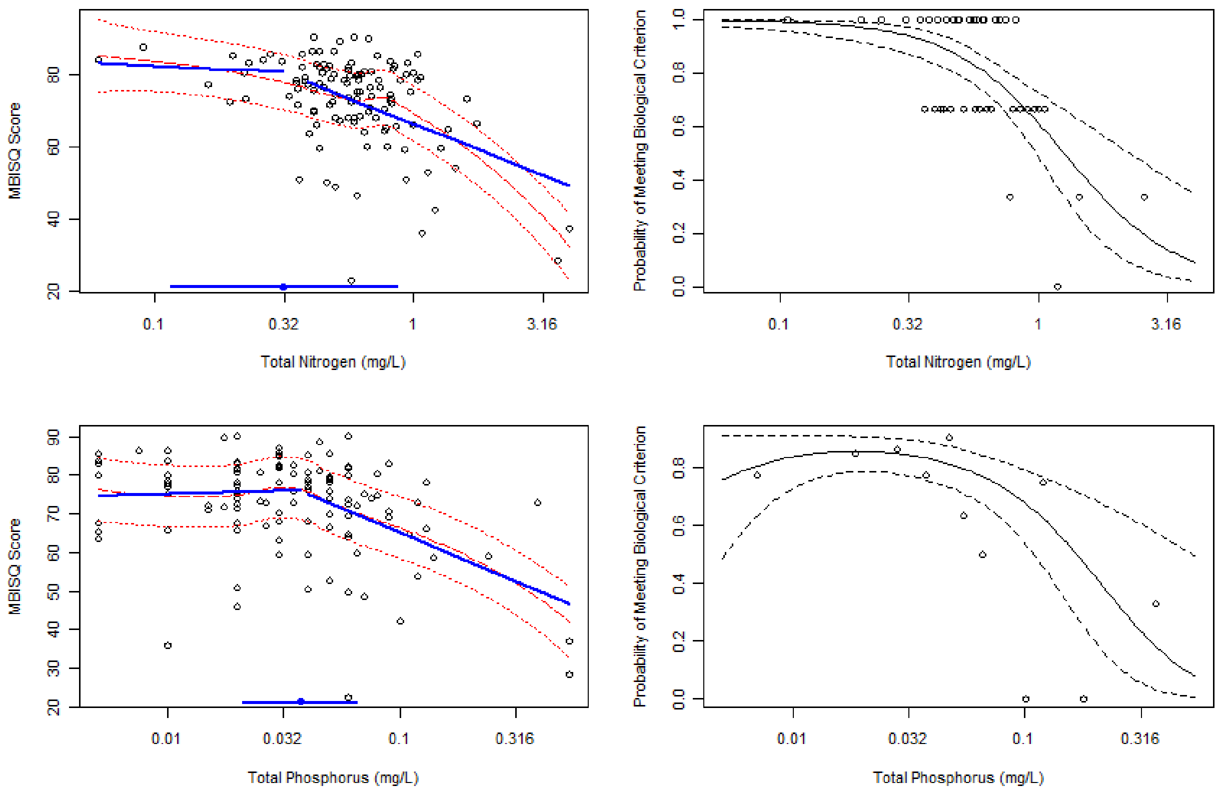


**Figure 7.4 Responses of M-BISQ08 score to nutrient parameters in Southeast bioregion. Curves on the left are raw data and on the right logistic probabilities. The solid black lines are LOWESS lines with confidence intervals. Blue lines are split point regressions and the mean change-point and 90% confidence intervals are shown as the blue points and horizontal blue lines, respectively, along the x-axis.**

**Table 7.1 Nutrient thresholds for each bioregion derived using change point analysis of raw M-BISQ scores (M-BISQ) as well as conditional probabilities (CP) of MBISQ scores being less than biological criteria using the revised MBISQ biological criteria.**

| | | TN | | | TP | | |
|---|---|---|---|---|---|---|---|
| | Response Variable | Median | Lower CI | Upper CI | TP | Lower CI | Upper CI |
| **East** | M-BISQ | 0.85 | 0.65 | 0.96 | 0.06 | 0.05 | 0.07 |
| **Southeast** | M-BISQ | 0.31 | 0.14 | 0.73 | 0.04 | 0.02 | 0.06 |
| **West_eco1** | M-BISQ | 1.23 | 0.64 | 2.37 | 0.12 | 0.05 | 0.3 |
| **West_eco5** | M-BISQ CP | 0.47 | 0.24 | 0.90 | 0.04 | 0.02 | 0.09 |

# 8 Scientific literature reviews to derive criteria

Additional scientific literature review and adjacent state criteria were discussed in detail in the original report (MDEQ 2009); the review has not been amended, and, for brevity, is not repeated here.

# 9 Magnitude, Frequency and Duration

## 9.1 Application of magnitude, frequency, and duration

The “magnitude” component represents nutrient concentrations demonstrated to be protective of the designated use. The magnitude may be interpreted as the average concentration for the target waterbody that will protect the designated use. For Mississippi streams, the magnitude of nutrients is represented by the thresholds developed here using various statistical approaches. While the magnitude component of nutrient criteria represents the long-term central tendency of the distribution, the frequency and duration components describe how often and by how much nutrient concentration can be above the central tendency while still being consistent with the reference distribution. However, criteria derived from distribution approaches have no direct link to cause and effect relationships with aquatic life uses. It can only be concluded that aquatic life uses will be maintained if the reference distribution is maintained. Therefore, the frequency and duration components are best established as an assessment test of whether the long-term distribution has shifted upward from the reference distribution; that is, test whether future monitoring data are consistent with the magnitude (long-term average).

A 25 percent annual exceedance probability is achieved if the assessment limit is set to the 75th percentile of the long-term average limit (magnitude component). That is, a 25 percent annual exceedance probability will only be achieved if the long-term average concentration remains at or below the magnitude target. Then a 10 percent Type I error rate (generally considered acceptable) can be used as a target to evaluate the frequency and duration to select nutrient concentrations. A binomial distribution can be used to estimate the frequency of exceeding a threshold given a number of trials (Figure 9.1). Figure 9.1 depicts the cumulative binomial frequency distributions for assessment periods (5 years) where the annual probability of exceedance is 0.25. A 10 percent type I error is achieved at the point where the cumulative probability of exceedance (probability of X or fewer exceedances) intersects 0.9; that is, the point where there is only a ten percent probability of observing greater than X exceedances in 5 trials. If we were to develop a frequency and duration test based on five year assessment period, then a level associated with an annual 25 percent probability of exceeding the magnitude target no more than twice during the five year period will achieve the target error rate. The 10 percent type I error rate (Figure 9.1) sums the probabilities of observing zero, one, or two exceedances given that p equals 0.25.

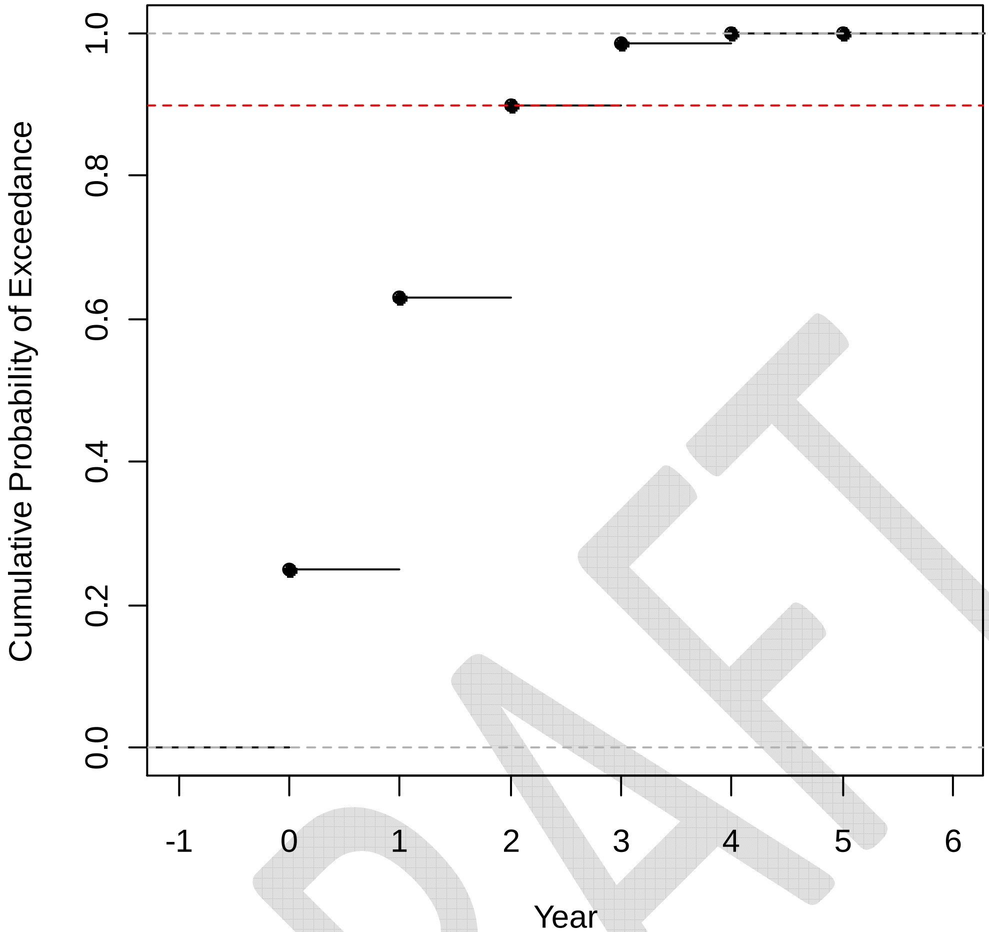


**Figure 9.1 Cumulative probability of exceedance in a five year monitoring network. The red dashed line indicates type I error rate when the annual 25% probability of exceeding the long term criterion is assumed.**

## 9.2 Variance Components

Because the goal of the criterion is to maintain a long-term average concentration, it is imperative to estimate the full range of variability within a waterbody given all sources of variance. In applying the water quality standards, the variability occurring in nature and the statistical variability inherent in sampling and testing procedures should also be evaluated.

Nutrient concentrations in streams typically follow or approximate a lognormal distribution. To evaluate the distribution, the geometric mean and standard deviation of the mean should be determined and an upper percentile (e.g.,75th) estimated. The mean is set to the long-term network 75$^{th}$ percentile of reference sites. The standard deviation has to be estimated from the variability observed within the baseline dataset. The approach effectively rescales the baseline dataset to a new central tendency equivalent to the magnitude component.

The Mississippi nutrient samples were collected across a range of spatial (more than 700 stations in the entire state) and temporal scales (2001 to 2009), replicated samples were also taken in select reference sites. In order to estimate the standard deviation (σ), the full range of variability or true inter-annual variance has to be estimated from annual, spatial, and within year (sampling) effects, such that the total annual variance is give as

$$S^2_{total} = S^2_{Station} + S^2_{Year} + Residual \qquad (1)$$

The station effect provides an estimate of the spatial variability within the region, while the year effect represents inter-annual variability. Inter-annual variability is largely driven by climatic and hydrologic cycles, as well as shorter timescale phenomena.


**Figure 9.2 Variance components of nutrient concentrations in Mississippi reference streams across different ecoregions. The annual standard deviation of the LnTN and LnTP were estimated using REML in R.**

We used a subset of data (65 stations sampled in 7 years in reference stations across the State) from the MBISQ database with replicate samples for nutrients to estimate the variance components. A Bartlett's test of homogeneity of variances indicated relative homogeneity of variances across ecoregions ($p>0.01$); therefore, we decided to analyze the components of variances across ecoregions as a whole. An ANOVA can be used to investigate the relative importance of various sources for variance on the long-term average of a sample distribution. However, due to highly unbalanced design of the dataset, we applied a restricted log-likelihood (lme in R nlme package) method to estimate the variance components of different factors. The results of such an analysis illustrate the importance of fully evaluating sources of variance across various spatial and temporal scales. Station, year, and within group effects accounted for different percentages of the overall variance in the long-term data set (Figure 9.2). Variances among stations have a much larger effect on LnTP than on LnTN. This pattern makes logical sense because the variability among individual stations for TP is most likely different among different ecoregions or small watershed levels in MS given the geological basis for P concentrations while variability among stations for TN is less different among regions in the reference stations reflecting a more ubiquitous, dominant atmospheric source. Results may be affected by short-term fluctuations in climatic, hydrologic, and biological conditions as well as sampling and analytical errors. The variances associated with site and year effects are based on concentrations that have been averaged over spatial or temporal scales and are thus less influenced by short-term events.

The true inter-annual standard deviation for a network of stations is calculated as:

$$\sigma_{tyr} = \sqrt{\sigma_{yr}^2 + \frac{\sigma_s^2}{n} + \frac{\sigma_{sd}^2}{n \cdot k}} \qquad (2)$$

where,
$\sigma_{tyr}$=true inter-annual standard deviation
$\sigma^2_{yr}$=the year to year variance (year effect)
$\sigma^2_s$=variance among stations (site effect)
$\sigma^2_{sd}$=within year variance for a station (date effect)
n=average number of stations within the network
k=the average number of annual samples collected at a station

The within year variance is calculated as the pooled within year variance across stations and years. Equation (2) applies if criteria are intended to be applied as a spatial average across a waterbody. The terms n and $\sigma^2_s$ are removed from the equation for criteria calculated based on and applied to a single location.

Nutrient annual criteria would depend on the site average criteria and the standard deviation. For TP, the standard deviation is determined 0.17; for TN standard deviation is 0.1.

So if a TP site average criteria for a region is 0.04, then the annual limit with 965% confidence would be exp(log(0.04)+0.34) = 0.056 mg/L.

# 10 Summary of recommended nutrient criteria

We revised nutrient thresholds using several approaches recommended by EPA that have been used by others to derive nutrient criteria for various states and regions and based on updated EPA guidance on stressor-response relationships (USEPA 2010). These benchmarks were based on reference approaches, stressor response approaches, and relevant literature values. The stressor response analyses were based on indirect invertebrate responses. The benchmarks derived from different approaches provided similar values of nutrient concentrations in various regions of Mississippi (Table 10.1.). In regions with relatively large sample sizes and available biological response data (e.g., East bioregion), TN and TP criteria were stronger due to a high degree of agreement among the different approaches and tight confidence intervals from these approaches. For regions with relatively small sample size and larger confidence intervals, we recommend a range of nutrient concentrations and recommend refining criteria when more data become available.

**Table 10.1 Summary of candidate criteria for each of the analytical approaches discussed. Values are central tendencies with confidence intervals in parentheses.**

| | **TN (mg/L)** | | | | |
|---|---|---|---|---|---|
| | **Modeled Reference (Land Use)** | **Distribution Based** | | **Stressor-Response** | **Literature/Other State Criteria** |
| | | **Least Disturbed** | **Biologically Attaining** | | |
| **East** | 0.360 (0.240-0.540) | 0.640 (0.600-0.700) | 0.740 (0.680 – 0.800) | 0.850 (0.650-0.960) | |
| **South Bluff** | 0.380 (0.170-0.450) | 0.460 (0.380-0.720) | 0.610 (0.450-0.760) | N/A | |
| **West** | 0.240 (0.160-0.370) | 0.780 (0.780-0.930) | 0.940 (0.940-1.110) | | |
| **West: North** | | | | 1.23 (0.640-2.370) | 0.180 – 2.000 |
| **West: South** | | | | 0.470 (0.240-0.900) | |
| **Southeast** | 0.380 (0.260-0.560) | 0.580 (0.540-0.710) | 0.680 (0.670-0.810) | 0.310 (0.140-0.730) | |
| | **TP (mg/L)** | | | | |
| | **Modeled Reference (Land Use)** | **Distribution Based** | | **Stressor-Response** | **Literature/Other State Criteria** |
| | | **Least Disturbed** | **Biologically Attaining** | | |
| **East** | 0.020 (0.010-0.040) | 0.050 (0.040-0.050) | 0.050 (0.040 – 0.060) | 0.060 (0.050-0.070) | |
| **South Bluff** | 0.070 (0.050-0.100) | 0.130 (0.080-0.160) | 0.110 (0.080-0.160) | N/A | |
| **West** | 0.030 (0.020-0.050) | 0.110 (0.090-0.140) | 0.100 (0.080-0.120) | | |
| **West: North** | | | | 0.120 (0.050-0.300) | 0.020 – 0.200 |
| **West: South** | | | | 0.040 (0.020-0.090) | |
| **Southeast** | 0.010 (0.010-0.020) | 0.030 (0.020-0.040) | 0.050 (0.040-0.050) | 0.040 (0.020-0.060) | |

## 10.1 Recommendations

The following recommendations were made as part of the original report (MDEQ 2009), but are still relevant and are repeated here.

- We strongly recommend that Mississippi start to collect phytoplankton and periphyton biomass samples (i.e., chl *a*) to help refine the nutrient criteria. Algae are direct indicators of nutrient enrichment and excess algae is a common problem associated with nutrient enrichment. Collecting and analyzing algal biomass will require minimum field and laboratory time and will strengthen nutrient criteria.

- We also recommend that Mississippi start to collect periphyton species composition samples. These samples can be preserved for a long time and can be analyzed upon funding availability. Periphyton species composition is a sensitive nutrient indicator and has been very useful for nutrient criteria development.

- Additional least disturbed sites in several regions should be identified and nutrient and macroinvertebrate data from these new sites should be collected to refine nutrient criteria. These regions include the South Bluff bioregion and the West bioregion ecogroup 5.

- Although we did not find a strong seasonal pattern of nutrient concentrations in streams, it was based on limited data for LD sites. Seasonal sampling of nutrients in LD sites would help to further explore seasonality and set criteria for nutrient criteria during different seasons.

- More sites and samples are needed to fully explore classifications and develop more defensible nutrient criteria for non-wadeable streams.

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